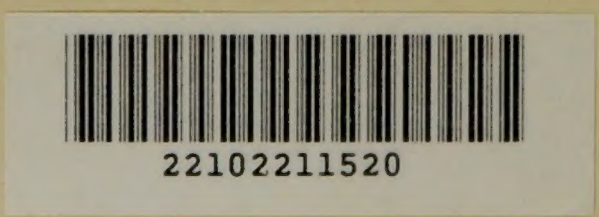
ANÆMIA

BIRCH AND LAZARUS



Murray Cowlie.
3. II. 1911.

B. G. Cowlie.
3rd Feb. 1911.

ANÆMIA

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BY

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PART I. VOLUME I.

NORMAL AND PATHOLOGICAL HISTOLOGY OF THE BLOOD

SECOND EDITION

(ENLARGED AND TO A GREAT EXTENT REWRITTEN)

BY

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WITH 5 ILLUSTRATIONS IN THE TEXT AND 5 COLOURED PLATES

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PREFACE TO THE SECOND GERMAN EDITION

It is now more than ten years since I, together with my pupil, A. Lazarus, published the First Edition of this part of *Anæmia*. Apart from a critical description of general clinical methods of examination, it dealt with the position at that time of the normal and pathological histology of the blood. It was, moreover, intended that this book should be especially devoted to a résumé of the various works published by me and my pupils, as well as the hitherto unpublished results of my investigations and the views which I had adopted on the basis of these results.

A glance over the hæmatological literature of the last ten years—a literature which has assumed almost immeasurable dimensions—shows that our *Anæmia* has stimulated investigation widely and has influenced it. The large majority of the communications have dealt with the problems which have been discussed in our book, and while some of these works support the views set forth, others oppose them. Histologists as well as clinicians have taken a very lively part in this criticism.

It has been of the greatest satisfaction to me that my seed has fallen on such fruitful soil; but it is especially pleasant for me to find that, after the completion of these ten years, the views which I have from the first defended as the foundation of modern cellular hæmatology, in spite of the opposition of many renowned observers, have been more and more acknowledged, and I am convinced that within a very short space of time absolute unanimity with regard to these questions will reign among hæmatologists, as far as the principles are concerned, in concord with my doctrines. It is true that more recent work has removed

many a stone from the building which I had erected, but, on the other hand, this same work has materially assisted in the completion of the structure. The foundation, however, has not been touched in any important detail.

This refers more especially to my doctrine of the anæmic conditions, which I have grouped according to the form of reaction of the bone marrow and to the doctrine of dualism of the white blood cells, which I have held from the very first. It is just with regard to this subject that many of my earlier pieces of evidence have been disproved by recent researches, and in many important details this question has assumed another aspect to that which it presented ten years ago. It is, however, with great satisfaction that I note from the recent literature, and particularly from the Transactions of the "Meeting of Scientists and Physicians," held in Cologne in 1908, that unanimity has been arrived at with regard to the chief points, and that the "unitarians" seem to have withdrawn from the fight.

It appears to be generally accepted now that the various granules of the cells must be regarded as products of specific metabolism. This has been demonstrated more especially in the more recent publications. In this connection the observations, for example, of Kollmann are very important. He showed that in the lower animals (*e.g.* the crab), a disappearance of the granules can be obtained by starvation. This observation may be able to throw much light on phenomena of human pathology in which the neutrophile cells have lost their granules either completely or in part. I have described this in a case of anæmia. Similar conditions have been met with in leukæmia, and the appearances of the blood in these cases have in many instances led to erroneous suppositions with regard to the genesis of the white blood corpuscles. If I might express a wish for the future, it would be that physiological chemistry should attempt to clear up the chemical nature of the granules, since it is possible that the substances involved might turn out to be of great interest for clinical, therapeutic purposes.

During the last five years we have been pressed both by our

publishers and by our readers to edit a new edition of *Anæmia*, but in view of the large number of questions which were still being discussed with energy, this was postponed—much, it must be admitted, against our desires. Pressing obligations have prevented me from taking part in the preparation of this Second Edition, and for this reason I have requested Privat-Dozent Dr. O. Naegeli of Zurich to utilize his well-known capabilities in assisting my original co-operator,—A. Lazarus,—to undertake the work.

P. EHRLICH.

FRANKFURT-AM-MAIN,
April 1909.

PREFACE TO THE ENGLISH EDITION

THE name of Ehrlich conveys two ideas directly to the mind of the ordinary student of medicine in England: blood and immunity. Of these two ideas, that of blood is the clearer in the mind of the average practitioner, because the teaching of the great German savant has reached him with more facility and in greater detail with regard to the histology of the blood than with regard to the intricacies of the mechanisms of side chains and antibodies.

The name of Ehrlich is one which the English hæmatologist and the English research student has learned to value, and one which he cannot regard as Teutonic; he claims it as an international name.

In introducing a work which has emanated from Ehrlich's school, and which in its first edition was in part written by Ehrlich himself, to the medical profession in England, no need exists for any recommendation of the author's. The value of the teaching of the greatest medico-biologist must be recognised by everyone.

The task of transferring the ideas of the authors into a readable form of English has been one which the translator has willingly attempted, because he holds the opinion that it will be of use to the medical profession in this country to have at its disposal a work which aims chiefly at defending the manifold doctrines which Ehrlich has introduced in hæmatology. Some of the views put forward may not be as widely accepted as the one author (Dr. O. Naegeli) would have the reader believe, but no attempt has been made to introduce any outside opinions into the work. It has been left entirely Ehrlichian.

The extraordinary application of intra-cellular chemistry

which characterises Ehrlich's blood work is well set forth in the pages of this book, and much of the material dealt with must be regarded as the result of genius working against the disadvantages of almost complete ignorance.

It is the earnest wish of the translator that his attempts to present Ehrlich's and Lazarus' *Anæmia* in a readable form to the British medical practitioner has succeeded, and that the English Edition may find the favour which the original edition undoubtedly enjoys.

H. W. ARMIT.

LONDON, *June* 1910.

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CHAPTER I

INTRODUCTION

DEFINITION—CLINICAL METHODS OF EXAMINING THE BLOOD

The term "anæmia" as it is applied in clinical medicine does not possess exactly the same meaning as the limitations which scientific investigation has imposed upon it would suggest. In the former certain prominent external symptoms are regarded as the characteristics of anæmic conditions: pallor of the skin and, as compared with the normal condition, a slighter degree of redness of the mucous membranes of the eyes, lips, oral cavity, and fauces. Not only is the existence of an anæmia deduced from the presence of these signs, but even the degree of the affection is measured by the extent of the symptoms.

It is obvious that a definition which is formulated on such a common and elementary symptom complex must include conditions which do not belong to it at all, while other conditions may perhaps be excluded which on account of their nature should be grouped with it. This fact is responsible for a number of uncertainties and contradictions.

It is therefore the first problem of a scientific consideration of anæmic conditions to carefully define their extent. The external symptoms mentioned above will be found to be little suitable for such a task, although it must be admitted that when applied in the proper place they are of practical importance.

The word anæmia in its etymological sense deals with a blood the content of which is smaller than in health. This abnormality

may be general and affect the whole organism, or it may be local and affect only a limited area or a single organ. The latter forms, the local anæmias, do not enter into the scope of the present work.

The blood content of an organism can clearly differ from that of a healthy individual in two ways: quantitatively and qualitatively. A diminution of the total quantity of blood, without any alteration of its composition, may be present; this is called *oligæmia*. On the other hand, the diminution of the quality of the blood may be absolutely independent of the total quantity, and must be recognised primarily by the diminution of those components of the blood which are physiologically most important. Accordingly the principal types of qualitative diminution of the blood, which are recognised, are the diminution of the hæmoglobin content (*oligochromæmia*), and the diminution of the red blood corpuscles (*oligocythæmia*).

All conditions in which a diminution of the hæmoglobin content is ascertainable must be regarded as anæmic. In the majority of cases, even if this is not quite constant, oligæmia and oligocythæmia exist simultaneously in varying degrees.

The most important methods of clinical hæmatology depend directly or indirectly on the recognition of these changes.

Up to the present no clinically applicable method for the determination of the total quantity of blood has been introduced. It is possible to a certain extent to utilise the observations mentioned above of the symptoms of redness or pallor of the skin and mucous membranes for this purpose. These symptoms are, however, dependent to a great extent on the composition of the blood, and not solely on the amount of blood contained in the peripheral vessels. In order, therefore, to utilise this means as a measure of the total quantity of blood, it is advisable to have regard to those isolated vessels which are visible to the naked eye, *e.g.* in the sclera. The most useful way, however, is to observe the size of the vessels in the fundus of the eye by means of the ophthalmoscope. Raehlmann has shown that in 60 per cent. of cases of chronic anæmia, in which the skin and

mucous membranes are pale, hyperæmia of the retina exists. This proves that the blood circulating in the vessels is paler but not sparser than normal. The character of the pulse also may give an important indication in cases in which the diminution of the quantity of blood is considerable; in marked oligæmia a very small and soft pulse is always found.

The way in which fresh wounds bleed may serve as a criterion of the quantity of blood; this, however, can only be utilised within certain limitations, depending to a large extent on the coagulability of the blood. Those who have had occasion to examine the blood of anæmic persons frequently will have experienced that this behaviour is subject to great variations. In certain cases scarcely a drop of blood can be obtained in the ordinary way, while in other cases the blood flows freely. There is little risk of mistake, if an absolute diminution of the total quantity of blood is assumed in the former case. The degree of fulness of the peripheral vessels is, however, only an index of relative value, since the blood content of the internal organs may be quite different.

The task of determining the exact quantity of blood in a body in figures has always been one of utmost importance. The solution would mark a very great advance in hæmatology. Of the methods which have hitherto been suggested for use in clinical medicine, that emanating from Tarchanoff deserves mention. Tarchanoff proposed that by determining the loss of water during profuse sweating, and by comparative red blood cell counts both before and after the sweating, an estimate of the quantity of blood could be arrived at. This method, apart from many theoretical difficulties, is much too complicated to be applicable to practice.

Quincke attempted to determine the quantity of blood by means of calculations, while carrying out therapeutic transfusion of blood. The quantity of blood in the person into whose vessels blood is being transfused can be calculated by means of a simple mathematical formula, from the number of red cells in his blood before and after the transfusion, and from

the quantity of the transfused blood and the number of corpuscles contained therein. But even this method is only applicable in certain cases, and is open to some theoretical objections. In the first place, it is dependent on the relative content of red corpuscles of the blood, inasmuch as the transfusion of normal blood into normal blood would not effect any change in the number. This suffices to show that this method is only of use in a few special cases. It has been demonstrated that an increase of red corpuscles in each c.mm. takes place in an individual with a very low red blood cell count, into whom normal blood has been injected, but it is extremely risky to attempt to determine the amount of the pre-existing blood from this calculation, since there is no doubt that the act of transfusion directly produces compensating currents of fluid and changes in the distribution of the blood.

The same objection may be offered to the suggestions recently made by Kottmann and Plesch. These observers injected physiological saline solution intravenously in large quantities, and after five minutes determined the total volume of red blood corpuscles (Kottmann) or hæmoglobin (Plesch) and compared the values gained with those derived from similar counts before the injections. Apart from the objections mentioned above, there are objections raised from the medical point of view against the introduction of such injections for the purpose of clinical examination. The injections are often followed by a considerable rise of temperature.

The method employed by Morawitz of determining the quantity of blood in an arm by means of a plethysmograph and of calculating from this the total quantity in the body seems to be quite worthless, in view of the local and temporary variations in the distribution of the blood, which cannot be ascertained but which are certainly considerable.

Haldane and Lorrain-Smith have attacked the problem from another standpoint and have used a comparatively simple method, which, however, is not quite free from objection on the point of safety. They caused their animals to breathe in a definite amount

of CO, then abstracted small quantities of blood and ascertained without difficulty the CO content of the total quantity of blood.

The results obtained in this way did not agree with those which had previously been accepted in physiology. According to Haldane and Lorrain-Smith, the relation between the total quantity of blood and the body weight in man varies between 1 : 16 and 1 : 30, while the average stands at 1 : 20·5, as compared with the hitherto accepted average value of 1 : 13. Douglas, however, compared Haldane and Lorrain-Smith's method directly with Welcker's by animal experiment, and found that the results tallied well.

Lorrain-Smith first applied this method for clinical purposes, and showed that in chlorosis there is always an increase in the total quantity of blood. The same author, working with M'Kisack, found the total quantity of blood in a boy aged 12 years, who was suffering from adhesive pericarditis with chronic cyanosis nearly twice as great as normal. Further communications have been made by Parkes Weber on experiments carried out by Haldane and Boycott and himself, and also by Örum. These observations showed that, in cases of megalosplenic and secondary polycythæmia, the quantity was two and a half to three times that of the normal quantity of blood.

There is no character of the blood that has been so carefully and frequently measured as the number of red corpuscles in a c.mm. of blood. The comparatively easy management of the counting apparatus and the guarantee of an apparently absolute measure have procured a ready acceptance of the counting methods for clinical practice.[1] Thoma-Zeiss's or similarly constructed apparatus are now in general use for the counting of blood corpuscles. It is presumed that the principles on which these apparatus are constructed and their methods of employment are known. A large number of fluids may serve to dilute the blood, all of which are capable of preserving

[1] For the determination of the proportions of white to red blood corpuscles, as well as of the various forms of leucocytes to one another, see the morphological section, p. 32.

the shape and colour of the red corpuscles, of preventing them from clumping and of permitting them to sediment rapidly. Pacini's and Hayem's fluids are the best known of these:

PACINI'S FLUID.

Hydrarg-perchloridi	2·0 grms.
Sodii chloridi	4·0 „
Glycerini	26·0 „
Aquæ dest.	226·0 „

HAYEM'S FLUID.

Hydrarg-perchloridi	0·5 grms.
Sodii sulphatis	5·0 „
Sodii chlorat	1·0 „
Aquæ dest.	200·0 „

The results obtained by these methods of counting are sufficiently exact for practical purposes, since, according to the researches of R. Thoma and I. F. Lyon, which have been confirmed by numerous observers, the experimental error is low. When 200 cells are counted it is 5 per cent., when 1250 cells are counted it is 2 per cent., with 5000 cells it is 1 per cent., and with 20,000 cells 0·5 per cent.).

As far as the practical application of the method is concerned, further considerations must be taken into account which exercise an unfavourable influence on the accuracy of the values. Cohnheim and Zuntz, *inter alia*, have shown that the blood in the larger vessels reveals a constant composition, but that in the smaller vessels and capillaries the corpuscular elements are subjected to considerable variations as to number, even in otherwise normal blood. For example, samples of capillary blood taken from both sides of persons suffering from hemiplegia do not contain the same number of corpuscles; while marked hyperæmia, cold, etc., increase the number of red cells locally. It is therefore necessary that blood taken for the purpose of making counts should be derived only from those portions of the body which are not subjected to marked variations, that all

procedures should be avoided which could alter the capillary circulation, such as violent rubbing, massage, and the like, and that the examination should be undertaken at a time of day when the number of blood corpuscles is not artificially influenced by the taking in of food or by medicaments.

It is usual to take the blood from the tip of the finger, and only when this is rendered inadvisable, *e.g.* when there is œdema of the finger, to select other situations, such as the lobule of the ear, the great toe (especially in children), etc. It is inexpedient to make a prick with a sharp needle or with a specially constructed open or hidden lancet; instead of all the complicated apparatus, the best instrument for the purpose is a new steel nib, one point of which is broken off, or a Sönnecken's vaccination lancet. The nib or lancet can be readily sterilised by heating in the open flame, and by their means a more suitable cut, rather than a prick, is obtained, from which the blood flows freely without the aid of marked pressure.

The material from which the countings of red blood corpuscles in healthy persons has been determined and published seems to be too extensive to deal with. According to the exact compilations of Reinert and von Limbeck, the following values (calculated for cubic millimetres and expressed in round figures) may be regarded as physiological:—

Males.

Maximum.	Minimum.	Average.
7,000,000	4,000,000	5,000,000

Females.

Maximum.	Minimum.	Average.
5,250,000	4,500,000	4,500,000

The difference between the two sexes only exists from the time of puberty in women. Up to the time of the onset of the first menstruation the number of red blood corpuscles is even a little larger (Stierlin).

The only other variation in the number of red blood corpuscles due to the age of the individual is found in the case of newly born infants, in whom polycythæmia is always present (up to 8½ millions during the first few days of life, E. Schiff). From the first taking in of food, however, the number decreases gradually, albeit in stages, until the normal is reached, which takes place in about ten to fourteen days. The oligocythæmia, which is occasionally observed during advanced age, is, according to Schmaltz, not a regular phenomenon, and should therefore not be regarded as a physiological peculiarity of senility, but must be ascribed to the manifold active circumstances which affect this age.

The influence which the intake of food tends to exercise on the number of red blood corpuscles must be ascribed in the main to the addition of water, and is so insignificant that the variations lie as a rule within the limits of error of the method.

Other physiological processes: menstruation (*i.e.* a single period), pregnancy, lactation—do not alter the number of blood cells to any appreciable extent. Nor are there any differences between the numbers in arterial and venous blood.

All the fluctuations in the number of blood corpuscles which lie within physiological limits are dependent on vasomotor influences (according to Cohnstein and Zuntz). Stimuli which cause a contraction of peripheral vessels lessen the number of red blood corpuscles *in situ*; stimulation of vasodilators, on the other hand, produces a reverse effect. This means that the physiological variations in the number in any given area is only an expression of an altered distribution of the red elements within the blood channels, and is quite independent of new formation and destruction of the cells.

Climatic conditions appear to have a great influence on the number of blood corpuscles. This matter is of equal importance

to physiology, pathology, and therapeutics, and has been the subject of lively debate since Viault called attention to it by his investigations on the heights of the Cordillera. His observations, as well as those of Mercier, Egger, Wolff, Koeppe, von Jaruntowski and Schröder, Miescher, Kündig, and others have shown that when a healthy man with a normal average count of 5,000,000 per c.mm. reaches a place situated considerably above the sea level, the number of his red blood corpuscles begins to increase. After from ten to fourteen days, during which time the number rises by stages, a new average value is reached and becomes constant. The height reached is considerably above the original value, and is in proportion to the difference between the height above sea level of the original and the new place of abode. Those who are born in high altitudes or who live there all their lives show a considerably higher average of the physiological number of blood corpuscles than persons living in lower lying situations, and this average is usually even higher than that of persons who have become acclimatised to the particular place or who merely stay at high altitudes.

The following scale gives an idea of the extent of the increase in the number of blood corpuscles over and above the normal average (5,000,000):—

Author.	Place.	Height above Sea Level.	Increase of
v. Jaruntowski	Görbersdorf	1,840 ft.	800,000
Wolff & Koeppe	Reiboldsgrün	2,296 ,,	1,000,000
Egger	Arosa	6,904 ,,	2,000,000
Viault	Cordillera	14,406 ,,	3,000,000

Exactly the opposite is observed when a person acclimatised to a high altitude, who shows these high blood corpuscle values, moves to a place situated at a lower level. Under these conditions the corresponding lower physiological average is gradually assumed.

These results have been confirmed in an enormously large number of observations of single cases. Some authors, however (Gottstein, Meissen, and others), have expressed the opinion that no material significance can be attached to them, on the ground that these counts depend on an illusion; since the capacity of the Thoma-Zeiss's cell is influenced by the alteration of the external atmospheric pressure. This objection has been finally disproved by Schaumann and Rosenqvist, and the values which have been recorded are now generally accepted as being the accurate expressions of the numbers of red blood corpuscles per c.mm.

At the same time, it must be pointed out that among those who really recognise the alteration in the number of red blood corpuscles a difference of opinion exists or existed as to the significance of these processes. Some observers were inclined to regard the variations in the number of blood cells as being wholly due to the action of vasomotor processes similar to those of which mention has been made above. In their opinion exposure to the sun's rays, differences of temperature, and the like are the most important factors. A. Loewy and his colleagues have shown that the number of blood corpuscles in the capillaries may vary in either direction to the extent of millions within the space of a few minutes under influences of this kind. Grawitz has expressed the opinion that the increase in the number of blood corpuscles is wholly explainable on the assumption of a marked concentration of the blood, resulting from an increased loss of water by evaporation from the body when existing at these heights. He was able to demonstrate that animals which were kept in correspondingly rarefied atmospheres behaved similarly. von Limbeck, Schumburg, and Zuntz opposed this argument with the objection, that if the loss of water could produce such a considerable raising in the number of cells a corresponding loss of weight must also occur, and this is not the case.

Schaumann and Rosenqvist have recently contended that the recorded increase in the red blood corpuscles at high altitudes is

the result of an actual new formation of these cells, and that a diminution in the number, occurring when the subject returns to lower levels, is caused by a destruction of the red elements of the blood. This contention can be supported by a large number of convincing facts.

In the first place, when a person moves to a high altitude the increase in the red blood cells does not take place at once, but frequently requires several weeks to develop. In the next place, the hæmoglobin value, the specific gravity, and the dry substance value of the blood alters simultaneously and in the same direction, although the changes are not always exactly proportional. Thirdly, as Koeppe first demonstrated, morphological changes occur in the blood cells. This observer noted that as a rule poikilocytosis and formation of microcytes took place immediately the subject arrived at the higher altitude. These changes are regarded as indications of the endeavour on the part of the organism to raise the respiratory surface of the total reservoir of hæmoglobin. Schaumann and Rosenqvist, A. Loewy and Franz Müller and also Foà, made the observation that when animals are placed under corresponding conditions a distinct increase of the blood-forming function of the bone marrow could be demonstrated histologically. The most convincing proof, however, was supplied by Jaquet and Suter, Abderhalden, and Loewy and Müller, in their experiments, which showed that rabbits and dogs which were kept for considerable periods at a height of 3000 or 6300 feet above the sea level, actually had a larger total quantity of hæmoglobin than the control animals which were kept at a lower level.

These facts justify the simple deduction, that under the influence of high altitudes a new formation of blood corpuscles and hæmoglobin actually takes place. Schaumann and Rosenqvist and Sellier, however, experimented further to determine which were the factors in connection with high altitudes which lead to the increase of the hæmatopoiesis, and concurrently arrived at the conclusion that diminution of the atmospheric pressure is the most important factor. The crucial experiment was performed

by A. von Koranyi and Bence, by which it was proved that the inhalation of oxygen inhibits the increase of the elementary constituents of the blood, and may even cause this change, after it has set in, to disappear.

The enormous amount of data dealing with this point justified an acceptation of the doctrine that life at high altitudes actually calls forth an increased production of blood, while the descent from a height to lower lying districts causes a destruction of the blood cells. On the other hand, it is much more difficult to decide (if this indeed be possible) what proportion of the changes which have been observed is dependent on vasomotor influences, and what proportion must be ascribed to true hæmopoietic processes. All that can be said at present is that the vasomotor influences are undoubtedly extremely active, and that both factors act simultaneously.

The influence of the tropics on the composition of the blood, and especially on the number of blood corpuscles, has been studied, as well as the influence of high altitudes. Both Eykmann and Glogner found that in spite of the fact that the pale appearance of Europeans in the tropics suggests some blood change, no such change can be detected. It appears in this connection also, that an alteration of the distribution of the blood without any quantitative changes is responsible for the pallor.

Thoma-Zeiss's and similar methods of counting blood corpuscles do not yield the same marked reliability with anæmic blood as they do with normal blood, in which the red corpuscles are all of the same size and all contain the same quantity of hæmoglobin. In anæmic blood, as will be shown later, the red corpuscles reveal considerable variations. Some of the cells are poor in hæmoglobin, while others are so small that they cannot even be seen when examined by the moist methods.

Slight differences in the individual blood discs are seen in the blood of healthy subjects when the examination is carried out in this manner. The physiological average of the diameter of the largest surface in men and women is 8·5 μ (maximum 9·0 μ

and minimum 6·5 μ; Laache, Hayem, Schauman, and others). The differences between the various elements become considerably more marked in anæmic blood. The average values must therefore be arrived at by noting the measurements of a large number of cells taken at random, and determining from such observations the maxima and minima. When the variations in the size of the discs are very great, microscopical measurements are of no scientific value.

Valuable as the knowledge of the absolute number of red blood corpuscles may be in forming an opinion with regard to the course of the disease, this knowledge does not throw any light on the hæmoglobin content of the blood, which is the real indicator of anæmia. A number of clinical methods serves the purpose of determining this value. These methods are either direct, as the colorimetric estimation of the hæmoglobin content; or indirect, as the determination of the specific weight, the volume of the red blood corpuscles, and even the determination of the dry substance contained in the total quantity of blood.

In dealing with the direct methods of estimating the hæmoglobin, which aim at measuring the intensity of the colour of the blood, and accept this as the index of the pigment content, mention must be made of a simple one. This method does not yield results of great accuracy, but is frequently found to be of value in permitting a rapid, rough idea of the state of affairs to be found at the bedside. The difference of colour between normal and anæmic blood can be more readily recognised when a drop of blood is allowed to fall on a piece of linen or filter paper, and to spread spontaneously, than when the drop is regarded as it issues from the prick in the finger. A little practice enables the physician to ascertain the degree of anæmia in this manner. If this simple and easily applied method were more used there is little doubt that the habit of resorting to the convenient diagnosis of anæmia in a number of doubtful cases would soon disappear. This method is further usually sufficient to convince neurasthenic patients, who believe that they are anæmic, and who look pale, that they are mistaken.

Tallqvist has constructed his "hæmoglobin scale" on this principle. By its means the hæmoglobin values may be roughly estimated in stages showing 10, 20, 30 per cent. and so on of hæmoglobin.

The most accurate apparatus for the measurement of the colour intensity of the blood is the Hoppe-Seyler's colorimetric double pipette.[1] This instrument contains an exactly titrated solution of carbon monoxide hæmoglobin, which has to be matched. The reliable preparation and conservation of this standard solution is, however, a matter of considerable difficulty, and for this reason the method cannot be included among those which can be applied clinically. It need not be considered in detail in this work. Zangemeister, a pupil of Kühne, has described an apparatus for colorimetric estimation, with which he has carried out a number of hæmoglobin determinations. The apparatus depends on the principle that the pigment content may be calculated from the measurement of the column of fluid, which has been matched as far as colour is concerned to a standard solution. Zangemeister employed a methæmoglobin glycerine solution derived from pig's blood as his standard solution. As far as the author is aware the clinical value of this method has not yet been tested. It is, however, highly desirable that this testing should be undertaken. The chromophotometer, which was described by Plesch and employed in physiology, is much too complicated for practical application.

The physician must therefore be satisfied for the present to work with less exact apparatus, in which tinted glass or more or less stable pigment solutions are employed for the purpose of having a coloured solution to which the colour of the blood is to be matched. Fleischl's hæmometer may be mentioned as one which depends on this principle, and Gower's hæmoglobinometer is also freely used in practice, being cheaper than others

[1] *Translator's Note.*—In this country Gower's hæmoglobinometer, with Haldane's carbon monoxide blood solution, is widely used, and yields results which are as exact as those obtained with Hoppe-Seyler's apparatus. The standard tube is sealed and retains its colour well, and is quite uniform. Each fresh tube bought, however, should be re-standardised by the clinician against a normal sample of blood, containing five million erythrocytes.

and yielding just as good results. Miescher's modification of Fleischl's hæmometer, apart from being more accurate, possesses an advantage over the original apparatus in that it records the absolute as well as the percentage values of hæmoglobin in the blood.

Sahli's hæmometer has acquired clinical popularity for good reasons. This instrument is provided with a test colour in the form of a permanent diluted solution of hæmatin chloride. The blood sample must therefore be taken in one-tenth normal hydrochloric acid, in order that the hæmoglobin may be converted into hæmatin chloride. It is unnecessary to enter into a detailed description of the method of using this apparatus, since each instrument is provided with minute details of the mode of use. The same also applies to the various apparatus mentioned above. Suffice it to mention that each Sahli's apparatus is now adjusted to a standard sample of healthy human blood containing 5,000,000 red blood corpuscles.

All these apparatus indicate what percentage of the normal quantity of hæmoglobin the sample of blood under examination possesses, and yield results which for practical purposes and as relative values are sufficiently accurate. In the hands of inexperienced observers, however, errors of 10 per cent. and more may occur (see K. H. Meyer).

Biernacki has raised objections to the colorimetric methods of estimating the hæmoglobin content quantitatively, on the ground that the colour intensity of blood is not wholly dependent on its hæmoglobin content, but is partly due to the coloration of the plasma and to the quantity of albumin contained in the blood. This objection, however, cannot be supported with regard to the estimation of the colour value by means of the apparatus mentioned, since the blood is diluted so considerably with water that any differences which may have originally been present become negligible.

Of the methods of determining the hæmoglobin content of blood indirectly, the one by means of which the pigment is calculated from the iron content of the blood would seem to

be quite accurate, since hæmoglobin possesses a constant Fe content of 0·42 per cent. The correctness of this may be admitted as far as normal blood is concerned; a definite proportion between hæmoglobin and iron content of the blood actually exists.

This method, however, is not to be recommended for determining the hæmoglobin in pathological blood. If the blood of an anæmic person be tested under the microscope with chemicals which give a reaction with iron, it will be found that many red blood corpuscles give the iron reaction. This would mean that iron has been detected which is not in the form of hæmoglobin. Iron may be present in the form of the albuminate of iron, which is not recognisable as such in the morphotic elements including the white corpuscles. Further, it is known that the iron content of all the organs of anæmic persons is markedly increased (Quincke), obviously as a result of the increased destruction of hæmoglobin ("sediment" iron, "spodogenous" iron). In the number of instances, consideration must be given to the fact that the therapeutic application of iron will increase the quantity of this metal in the blood and tissues.[1] The foregoing will show that any attempt to calculate the hæmoglobin content from the iron content in pathological conditions is unreliable.

This discursion was considered to be necessary on account of Biernacki's work, inasmuch as the procedure of deducing from the iron content the hæmoglobin content of the blood has led to many curious results. For example, in two cases of mild and one of severe chlorosis he found the iron content quite

[1] *Translator's Note.*—It is extremely doubtful whether this argument can be regarded as good. Experimental evidence exists that iron given by the mouth is not utilised by the organism to build up hæmoglobin; and further, that very little if any iron in inorganic combination is absorbed from the intestine. More than this, the translator was able to show that iron, existing in the form of free cations, exerts a highly toxic action on the tissues, and causes death in doses of a few milligrammes per kilo body weight. Considering the number of grammes of iron taken as medicine, it must be assumed that whatever small proportion is absorbed it is rapidly converted into complex iron combinations, and cannot accumulate in reasonable quantities in the blood.

normal. From this he argued that in chlorosis and other forms of anæmia there is no diminution, but rather a relative increase of hæmoglobin, and that the other albuminous components of the blood are diminished. Even if these iron determinations could be shown to be free from error (and it must be pointed out that they have been directly contravened by other authors), what has been said above will suffice to prove that the far-reaching deductions which Biernacki has drawn from his results are untenable. It must further be pointed out that delicate analyses like those on which Biernacki based his conclusions could only be accepted if confirmed by control experiments.

The number of erythrocytes[1] and the hæmoglobin value stand in close relationship to one another, but there is no complete parallelism between them. In certain pathological conditions the hæmoglobin content is higher than the number of red blood corpuscles would suggest, while in others it is lower. In some cases the diminution of the number of erythrocytes corresponds exactly to the diminution of the hæmoglobin value. For example, in a man a red cell count of four millions would correspond to a hæmoglobin value of 80. But in chlorosis, *inter alia*, the diminution of the hæmoglobin is greater proportionately than the diminution of the red cells, so that a reduction to 80 per cent. of red cells might exist simultaneously with a reduction to 60 per cent. of hæmoglobin. If the hæmoglobin percentage be divided by the red cell percentage, the quotient may be regarded as the expression of this relation. This quotient is called the "blood corpuscle value" or the "colour index." In the instance cited above it would be $\frac{60}{80} = 0{\cdot}75$. The colour index may be even greater than 1, when the diminution of the hæmoglobin is smaller than that of the erythrocytes. This occurrence was first observed in progressive pernicious anæmia. According to Meyer and Hieneke,

[1] *N.B.*—The term "erythrocyte" is not usually employed by English writers, but it is so handy that use will be made of it in this work.

the colour index of the fœtal blood is normally greater than 1, *e.g.* in the fifth month it is 1·6 and in the seventh month it is 1·4.

The disturbance of the parallelism between the number of blood corpuscles and the hæmoglobin content is dependent both on the changes in size of the red blood cells and on the fact that the individual blood discs may be either poor in hæmoglobin or anæmic, or under certain conditions they may be rich in hæmoglobin and appear as cells of an embryonal type in the blood

It thus becomes clear that it is not permissible to regard the number of erythrocytes in a given sample of blood as an independent indicator. It can only be of importance when considered in its relations to the result of the determination of the hæmoglobin and of the histological examination. In connection with this matter, it is necessary to call attention to the fact that a not inconsiderable source of error in the counting of erythrocytes in pathological cases depends on the failure of the observer to recognise and count the smallest forms of cells, when using the objectives which are usually employed for the purpose.

For this reason it is at times desirable to supplement the record of the number of red blood corpuscles by a determination of the size of the individual cells. This is carried out directly by measuring the diameter by means of an ocular-micrometer, which may be applied both with dried and with moist preparations, although the former are preferable on account of simplicity. The carrying out of this method, however, necessitates special care with regard to technique. It will be noticed that the red blood corpuscles in normal blood when lying in a thick layer appear smaller than when lying in a thin layer in a dry preparation. This difference is due to the fact that in the thick layer the red discs float about in the serum before drying, while in the thin layer they are connected to the surface of the slide by means of a capillary layer of serum. The drying takes place almost instantaneously in the latter case, the process starting from the

periphery of the disc, so that a change in the shape or size of the cell cannot occur. The process of drying in thick layers takes place more slowly, and is therefore accompanied by a shrinking of the discs (see p. 12 for figures).

The examination of the specific gravity of blood has always been regarded as highly important, since the number of corpuscles and the hæmoglobin content can be calculated from the measure of the density of the blood. There are two methods which do not necessitate elaborate apparatus and which are not too complicated for practical clinical purposes for the carrying out of these estimations, and fairly extensive data have been collected by both. The one has been devised by R. Schmaltz, and consists in weighing exactly small quantities of blood contained in glass capillaries (capillary-pycnometric method), while the other was worked out by A. Hammerschlag, based on the principle described by Fano, and consists in finding out the proportions of a mixture of chloroform and benzole, in which a drop of the blood to be examined neither floats nor sinks, *i.e.* which possesses exactly the same specific gravity as the blood.

According to the investigations of these authors and of a number of others who have used their methods, the specific gravity of the blood as a whole under physiological conditions is from 1058 to 1062, or on the average 1059 (in women 1056). The specific gravity of the serum is 1029 to 1032, or on the average 1030. From these figures it becomes clear that the material weight of the blood must be largely caused by the red blood corpuscles. If the erythrocytes are reduced in number, or if, while their number remains at the normal level, they become less in volume, or lose part of their hæmoglobin, the specific gravity will be found to be correspondingly reduced. In all anæmic conditions a diminution of the specific gravity of the blood must therefore be expected. And conversely, an increase in the number of red cells and a rising of the hæmoglobin value will be associated with an increase of the density of the blood.

Schmaltz was the first to show that the correspondence between the specific gravity and the hæmoglobin content of the

blood was much closer than that between the specific gravity and the number of red blood corpuscles. This correspondence is so constant that Hammerschlag expressed it in the following tabular form—

Specific Gravity.	Hæmoglobin Content (Fleischl).
1033–1035	25–30 per cent.
1035–1038	30–35 „
1038–1040	35–40 „
1040–1045	40–45 „
1045–1048	45–55 „
1048–1050	55–65 „
1050–1053	65–70 „
1053–1055	70–75 „
1055–1057	75–85 „
1057–1060	85–95 „

Dieballa, who has also studied this question closely, was able in part to confirm Hammerschlag's results, and in part to supplement them. He deduced from his comparative estimations an average value: differences of 10 per cent. in the hæmoglobin value (Fleischl) correspond to rough differences of 4·46 per mille in the specific gravity (measured by Hammerschlag's method). It was shown, however, that variations in the specific gravity up to 13·5 per mille are met with without any alteration in the hæmoglobin content, and it was found that these variations were greater in cases in which the hæmoglobin value was high. There are regular differences between the blood of men and that of women; in the latter the specific gravity is from 2 to 2·5 per mille lower with the same hæmoglobin value. When the parallelism between the number of erythrocytes and the hæmoglobin content is markedly disturbed the influence of the stroma of the red corpuscles on the specific gravity of the blood becomes recognisable. Dieballa calculated that the stroma may cause a difference of from 4 to 5 per mille in the specific gravity of two samples of blood having the same hæmoglobin values.

In this way it will be seen that the determination of the specific gravity may suffice in many cases for the purpose of determining the relative hæmoglobin content in a sample of blood. In nephritis and disturbances of the circulatory organs, and in leukæmia, however, the correspondence between the specific gravity and the hæmoglobin content are masked by extraneous conditions.

The physiological variations in the specific gravity of the blood of one and the same individual, which are due to the intake of fluid and the excretion of the same, does not exceed 0·003 (Schmaltz). These variations must correspond to those which affect the hæmoglobin content and the number of blood corpuscles, and must occur under conditions similar to those which produce the last-named variations.

A number of investigations, including more particularly those of Hammerschlag, von Jaksch, von Limbeck, Biernacki, Dunin, E. Grawitz, and A. Loewy have avoided an omission of which the earlier workers were guilty, by determining the specific gravity of at least one of the constituents (the blood corpuscles or the serum) as well as that of the blood as a whole. These observations all showed that the red corpuscles were responsible for the variations of the specific gravity of the whole blood. The changes in the cells were in part variations in their number or changes in their distribution, and in part due to their chemical lability: loss or gain in the water content, variations in respect to the albumin content, etc. The fluid of the blood possesses much greater constancy. No material difference appears to exist between the serum and the plasma (Hammerschlag). Even in severe pathological conditions, in which the blood as a whole is found to be specifically much lighter than normal, the serum retains its physiological composition, or only shows slight variations in concentration. Marked lowering of the specific gravity of the serum is far less often observed in actual diseases of the blood than in chronic renal diseases and disturbances of the circulation. E. Grawitz, however, has stated that certain anæmias, especially those following hæmor-

rhage and those following wasting conditions produce a recognisable depression in the specific gravity of the serum. Even if these contentions appear to be somewhat contradictory, it follows as a result of these observations that it is necessary in carrying out a scientific examination of the blood to examine the specific gravity of the serum or of the corpuscles in all cases, as well as that of the blood as a whole.

A method which is closely related to the determination of the specific gravity of the blood is that which aims at the determination of the dry substance of the blood, called hygraemometry, and which has been introduced into clinical use by Stintzing and Gumprecht. This examination is capable of supplementing the methods already described in a useful manner, since it can be carried out in practice with small quantities of blood, which can be obtained at all times and under all conditions. Small quantities of blood are collected in minute weighing bottles, weighed, dried for twenty-four hours at 65° to 70° C., and then weighed again. It appears that the values obtained from these weighings of the dry substance possess certain peculiar importance, since they do not always give results which stand parallel to the specific gravity, the hæmoglobin content, or the number of blood corpuscles. The normal value for men is 21·6 per cent. and for women 19·8 per cent.

A further procedure which admits of indirect deduction with regard to the hæmoglobin values of blood is the estimation of the volume percentage of the red blood corpuscles in the total volume of blood. In order to carry out this estimation it is necessary to have a method of separating the corpuscles from the fluid of the blood, without materially altering the composition of the blood. The older methods do not fulfil these conditions, for they either depend on defibrination of the blood, a process which is not possible even with the quantity of blood available in the clinic, or on the addition of sodium oxalate or other substance which inhibits the coagulation of the blood. The separation of the two components of the blood was achieved either by simple sedimentation or by centrifugation by

means of a special centrifuge, constructed by Blix-Hedin and Gärtner, called the hæmatocrit.

Even the manifold dilution fluids which are used for these methods of examination, such as physiological saline fluid, 2·5 per cent. solution of potassium bichromate, etc., cannot be regarded as being indifferent as far as the volume of the red corpuscles is concerned (H. Koeppe). A solution which does not alter the cells at all would have to be specially adapted to each sample of blood. For this reason the method suggested by M. Herz deserves consideration, in which the coagulation of the blood in the pipette is prevented by rendering the walls absolutely smooth by means of cod liver oil. Koeppe has modified this method slightly. He uses a suitably constructed pipette,[1] and after cleaning it carefully fills it with cedar-wood oil. When full he sucks up the blood as it issues from the prick in the finger. The blood ascending in the pipette pushes the column of oil before it, and inasmuch as it only comes into contact with perfectly smooth walls it remains fluid. The oil, being lighter than the blood, is then separated from the latter by means of a centrifuge which is specially adapted for the purpose, and at the same time the plasma is separated from the blood corpuscles. In this way three sharply defined layers are formed, the upper oil layer, the middle, plasma layer, and the lower the layer of corpuscles. As the apparatus is calibrated, the relative volume of the plasma and corpuscles can be read off. It is not possible to detect any changes in the corpuscles microscopically.

Although it may be admitted that this procedure appears to be technically difficult to carry out, it remains the only one available up to the present which fulfils the chief requirements of clinical pathology in this respect. The results which Koeppe has obtained up to the present, but which are not very numerous, show that the total volume of the corpuscles vary between 51·1 and 54·8 per cent. (average, 52·6 per cent.).

M. and L. Bleibtreu have attempted to determine the relative

[1] Manufactured by Hugershoff, Leipzig.

volume of the red blood corpuscles to the plasma indirectly. They mixed blood with varying quantities of physiological saline fluid, and determined in each mixture the nitrogen content of the fluid, which they separated from the cells by sedimentation. From these results they calculated mathematically the volume of the serum and of the red blood corpuscles. Apart from the fact that this method necessitates a dilution of the blood with physiological saline fluid, it is far too complicated and requires too large a quantity of blood to justify its application in clinical medicine. Th. Pfeiffer has attempted to introduce it into clinical use in suitable cases, but so far has not obtained definite results. It can, however, be shown that the relation between the volume percentage of the red corpuscles and the hæmoglobin content is not constant. For example, in acute anæmias, an "acute" swelling of some of the red cells has been observed by M. Herz, so that a corresponding increase in the total volume results, without any increase in the quantity of hæmoglobin taking place. This deduction has received support from the observations of von Limberg, Gerhard, and others, who found that in catarrhal jaundice the red cells undergo a considerable increase in volume under the influence of bile salts.

As has been stated before, the most valuable indicator of the severity of an anæmic condition is to be obtained by the estimation of the hæmoglobin content of the blood. Those methods of examination, which yield neither direct nor indirect information with regard to the hæmoglobin content, are only of importance on account of the possibility of throwing light on the special pathogenesis of the individual diseases of the blood.

A very large amount of work has been carried out by excellent investigators, especially physiologists, with regard to the determination of the reaction of the blood.

The alkaline reaction of blood cannot be demonstrated by allowing litmus paper to be moistened by fresh blood on account of the colour of the blood itself. Specially sensitive litmus paper is well moistened with dilute salt solution, and the

blood is then allowed to flow over the paper; and lastly, this is washed off with more salt solution. It is very difficult to determine the exact degree of alkalinity. It is sufficient for the present work to state that all the newer methods depend on a laking of the blood and subsequent titration with normal tartaric acid solution against resorcin blue paper (litmoid). In general a fair quantity of blood is required for this purpose, *e.g.* 5 to 8 c.c. C. S. Engel, however, has constructed an alkalimeter, by means of which the reaction can be determined with $\frac{1}{20}$ c.c. of blood.

As matters stand at present the clinician must be warned against the introduction into practice of uncertain methods of examination which yield varying results. The very fact that the results are expressed in figures awakens a false appearance of accuracy, which should be avoided even more than the utilisation of subjective methods of examination.

The doctrine, which has been built up on the basis of the methods referred to, with regard to the alkalinity of the blood and the deductions which have been drawn from this doctrine, have been attacked in a very convincing manner by Friedenthal. Friedenthal showed that the method which is almost always employed of determining the reaction by means of litmus is absolutely unsuitable, since litmus displaces carbonic acid from the carbonates, and in this way the alkali is set free. If the test is carried out with phenolphthalein, a neutral reaction of the blood can be clearly demonstrated. He suggests that all the biological actions and phenomena which have been ascribed to the alkalinity of the blood and blood serum may be explained by ferment action. He even states that it can be shown that artificially produced alkalinity of the blood, even when very slight, prevents these biological actions.

The investigations undertaken by Brandenburg deserve special mention. These show that it is necessary to distinguish between the alkali which is combined with albumin and that which is combined with carbonic acid. These two combinations can be divided from one another by dialysis, since the latter passes

through a membrane, while the former, which is firmly bound to the proteid molecule, is prevented by the colloid from dialysing. He was able to show that the dialysable portion of the alkali represented a very constant value (corresponding to about 60 mgrms. of NaOH), while the non-dialysable portion was subjected to considerable variations. It is particularly striking that under pathological conditions, even when considerable alterations of the total alkalinity were present, the amount of dialysable alkali—"the alkali tension"—remained practically unaltered.

A determination which will probably receive greater attention in the future than it has in the past from clinicians is that of the rapidity of coagulation. Results which can be compared with one another may be obtained by means of the handy apparatus constructed by Wright, and called the coagulometer. In certain conditions, especially in the acute exanthemata and in the various forms of hæmorrhagic diathesis, the rapidity of coagulation of the blood is distinctly diminished. The coagulability may even be abolished. At times, on the other hand, a definite hastening of the coagulation in comparison to the normal can be determined. Wright has shown, in his excellent investigations, that the coagulability can be influenced by drugs; calcium chloride and carbonic acid increase the coagulability; while citric acid, alcohol, and increased respiratory movements diminish it.

Under normal conditions the blood coagulates in from three to twenty minutes. Under pathological conditions the coagulation may be delayed for half an hour to one hour, and at times, as in hæmophylia, even to eight to ten hours (Hayem).

Hayem has repeatedly called attention to a condition which may possibly bear some relation to the coagulability of the blood. In spite of the coagulation of the blood having taken place, under certain conditions the separation of the serum from the clot only occurs to a slight extent, or it may be entirely absent. Hayem states that he has noticed this behaviour in the blood of patients suffering from purpura hæmorrhagica, protopathic pernicious anæmia, the cachexia of malaria, and some infectious diseases.

Large quantities of blood are required for these observations, such as are not frequently to be obtained in practice. Certain precautions which have been found to be of use in the preparation of diphtheria antitoxin must be adopted, in order to obtain as large a yield of serum as possible. These consist in the collecting the blood in longish vessels, which have been previously thoroughly cleansed, and more especially freed from all traces of fatty substances. If the clot does not retract spontaneously it must be separated from the wall of the vessel, without damaging it, by means of a flat instrument, like a paper knife. If no separation takes place in the cold it is always possible that better results may be obtained in the incubator.

In spite of all care and the application of all the contributory means, it sometimes happens, especially when pathological conditions are present, that not a trace of serum can be gained from a fairly large quantity of blood. Ehrlich obtained only about 100 c.c. of serum from 22 kilos of blood, from a horse which had previously been immunised against diphtheria and had yielded extraordinarily large quantities of serum. The horse had been bled to death on account of a tetanus infection.

It is quite possible that this condition may claim the attention of clinicians in future. Hayem has attempted to distinguish protopathic pernicious anæmia from other severe forms of anæmia by means of an abnormal separation of serum. He is further of opinion that a bad prognosis is justified in cachectic conditions when this phenomenon is met with.

Mention must be made of some further methods, by means of which the resistance of the red blood cells toward external noxes of various kinds can be tested.

Landois, Hamburger, and von Limbeck determined that concentration of a salt solution ("isotonic concentration" Hamburger) in which the red blood corpuscles are preserved, and that which causes the hæmoglobin to issue from the stroma. They found that the lower the concentration of the salt solution is, in which the erythrocytes still remain unaltered, the higher is their resistance.

Bettmann has employed Lugol's solution in varying concentrations with great advantage in these determinations, since the influence on the erythrocytes exerted by the salt solution can be controlled very clearly under the microscope. Rosin and Bibergeil have experimented with various stains, including methylene-blue and neutral red, with a similar object.

Laker has tested the erythrocytes as to their power of resistance toward electric discharges from Leyden jars, and has measured this resistance by recording the number of shocks which can be passed through the sample of blood without producing a damage to the cells.

Rosin and Bibergeil have noticed that fresh blood of healthy persons enclosed in the moist chamber, without the addition of any chemicals or the application of other extraneous means, retains its morphological components in an intact condition considerably longer than the blood of anæmic persons does. They consider that this is evidence of the smaller resistance of anæmic as compared with healthy blood.

The study of the hæmolysins has proved itself to be of special importance to this question. H. Sachs' experiments with the poison of the cross spider have revealed that the blood of chickens which have just been hatched is absolutely insensitive toward arachnolysin, while the blood of adult hens is extremely sensitive toward this substance.

In order to obtain a true conception of the resistance of the erythrocytes, it would naturally be necessary to test the effect not only of every possible mixture, but also of every form of physical, mechanical, thermic, and other stimulus. It might be found that a special kind of erythrocyte A, which shows a much smaller degree of resistance toward a certain chemical substance than a second kind of erythrocyte B, would behave in an absolutely similar manner toward another form of stimulus.

Clinical medicine has not benefited materially up to the present by these methods. One thing, however, is certain, namely, that in some diseases, such as anæmia, hæmoglobinuria, and after

some forms of poisoning, the resistance of the red blood corpuscles is ascertainably reduced.

Closely related to these methods, which only affect the red blood corpuscles, is the method of determining the freezing-point depression of the blood as a whole and of the serum. This is known as cryoscopy.

The cryoscopy of the blood is carried out in the same manner and with the same apparatus as that of the urine. The information which has been gained with regard to the molecular concentration of the blood is extremely meagre, and has not yielded any valuable results, more especially for the pathology of the blood. At all events, this method has not taught anything which had not been ascertained previously by means of the determination of the specific gravity.

CHAPTER II

THE MORPHOLOGY OF THE BLOOD

A.—METHODS OF EXAMINATION

A GLANCE through the history of the microscopy of the blood shows that this has been divided into two epochs. In the first, which has been distinguished especially by the work of Virchow and Max Schultze, a number of positive facts were rapidly collected, and the various forms of leukæmia were recognised. But after this no further progress was made for several decennia. This stand-still was due to the fact that the observers limited themselves to the study of the blood in its fresh condition. All that was to be seen with the assistance of these simple means had very soon been thoroughly exhausted by these excellent observers. That these methods were insufficient can best be shown by the history of leucocytosis, which according to Virchow's teaching was supposed to be brought about by an increased production of cells by the lymphatic glands. The same is seen in the fact that leucocytosis and early leukæmia were not sharply differentiated, and the diagnoses were made on the basis of simple numerical determinations. It was only after Ehrlich had introduced the new method of examining stained dry preparations that the histology of the blood entered on its second era. We are indebted to Ehrlich for an exact differentiation of the various forms of white blood corpuscles, for a rational definition of leukæmia, for the knowledge of polynuclear leucocytosis, for the knowledge of the signs of de- and re-generation of the red blood corpuscles, and for their breaking down in hæmoglobinæmic processes. The same means of advance have obtained in the subject of the microscopy of the blood as have

been witnessed in the other departments of normal and pathological histology; improvement in technique must first be achieved before material advances in knowledge can be arrived at. For this reason it is difficult to understand how certain observers still recommend the old methods, and claim that a diagnosis can be made in all cases from the examination of fresh blood specimens. It may be admitted that this would not be surprising, since the most important points have already been explained by means of the new methods. But the recognition of the more difficult cases, *e.g.* certain rare forms of anæmia, and for the recognition of definite kinds of cells, such as myelocytes, mast cells, etc., stained preparations are indispensable, as every experienced hæmatologist knows. The object of the examination, moreover, is not to facilitate a rapid diagnosis, but rather to enable an exact study of the details characterising the blood to be carried out, which cannot be ascertained from fresh specimens. It may with safety be claimed that at present it is possible to see all the characteristics of the blood, save those of the formation of rouleaux and of the amœboid movements of the white corpuscles, in stained dry preparations as well, if not better, than in the fresh specimens; while it must be admitted that there are many important details which can only be rendered visible by means of staining fixed preparations, and which remain invisible in fresh specimens.

Examination with the assistance of the so-called "dark field illumination" forms a valuable extension of the methods of studying the blood, especially in its fresh condition. This was first employed for this purpose by Dietrich. It has become possible with its assistance to demonstrate certain morphological details, *e.g.* shadows in the corpuscles, more clearly than had been done previously, while even some biological processes, such as hæmolysis, can be rendered directly visible by means of the dark field illumination. The special morphology of the blood cell, however, has not been advanced by this method, nor by the method of examining with the aid of ultra-violet rays (Grawitz and Grüneberg).

As far as the purely technical or practical aspect of the

question is concerned, the examination of stained dry films is undoubtedly much more convenient than that of fresh specimens. The former enables the observer to be independent with regard to place and time. Fixed specimens may be put aside for months without any special precautions, and then studied closely under the microscope. The examination of one specimen can be continued for any length of time, and can be repeated at any future date. On the other hand, the examination of the blood in the fresh condition is only possible at the bedside, and must be completed rapidly, since the blood changes quickly, by clotting, by destruction of the white cells, etc., so that an exhaustive study cannot be undertaken at all. An additional advantage of the former consists in the fact that the making and staining of dry blood films may be regarded as one of the easiest and most convenient of all the methods of clinical histology. It is therefore advisable to describe the technique in detail in this place, in order to awaken wide interest for this mode of examination.

It is further found advisable to describe in this place the application of stained dry specimens for the determination of the important numerical relation between the red and white blood corpuscles, and of the proportional percentages of the various forms of white corpuscles.

It is absolutely necessary that the investigator should be able to make a perfect, uniform film. Quadratic ocular diaphragms (Ehrlich-Zeiss) are essential.[1] These either represent a complete series, so that the sides of the square having measurements in the ratio of 1 : 2 : 3 . . . 10, would give segments of the field in the ratio of 1 : 4 : 9 . . . : 100, or take the form of the more handy eye-piece, devised by Ehrlich and constructed by Leitz, which possesses a neat mechanical appliance by means of which a centrally situated, square segment of the field of a desired size can be interposed. The eye-piece is used in the following manner. A normal blood preparation is examined by first counting the white blood corpuscles as seen in the field when a No. 10 dia-

[1] These square eye-pieces are not frequent in this country.—(The Translator).

phragm (or the segment 100 of the eye-piece) is interposed. Next the diaphragm No. 1 is interposed so that only one-hundredth part of the field is exposed, and in this field the number of red blood cells are counted. This is done without shifting the specimen. Next, another part of the specimen is chosen at random, and the count repeated; in each case only one hundredth or one twenty-fifth of the field employed for the white cell count is used for the red cell count. About 100 such counts are made in each specimen. The number of red cells is then multiplied by 100, and compared to the number of white cells counted. When the white cells are very numerous and the counting in a large segment becomes difficult, a smaller diaphragm is employed, such as 81, 64, 49, and so on.

The important determination of the percentage proportions of the various forms of leucocytes is carried out by noting the numbers of each kind in a series of several hundred cells. This can be done by an experienced individual in less than a quarter of an hour.

(*a*) Making a Dry Specimen.

For the purpose of making perfect dry films, it is of especial importance to use cover-glasses of a particular kind. The cover-glasses should not be thicker than 0·08 to 0·10 mm.; the glass should not be brittle, it should have no defects, and it must be of such a quality that it will bend to a considerable extent without breaking. The slightest roughness of the glass renders it useless for the purpose. The cover-glasses must be subjected to a scrupulous cleansing, and must be absolutely freed from all traces of fat. For ordinary purposes it is sufficient to immerse the glasses in aether for thirty minutes, without allowing them to overlap, and then to wipe them with an old soft linen or cambric cloth. They are then dipped into alcohol for a few minutes, and are again dried in the same manner as they were after immersion in aether. They should then be placed in a dust-tight glass bottle or box until required. The fact that these cover-glasses are cut out of a large cylinder and not out of a flat plate of glass shows

that they are the only kind which would allow of the formation of a capillary space between them when superimposed on one another, in which the blood can spread out spontaneously. The slightest unevenness or brittleness of the glass would render it impossible for the curve in the one to correspond sufficiently exactly to the curve in the second. It is only when the glasses are of this quality that it becomes possible to slide the one from the other without using such force as would destroy the film.

The cover-glasses must be held by means of forceps [1] in order to avoid any soiling, and especially any contamination of the blood by moisture of the finger. The lower glass is best held in a pair of forceps provided with a catch and broad flat points (*a*). The inside of these points can be lined to the extent of about 3 cms. from the tips with leather or English blotting-paper. The other cover-glass is best held in a pair of forceps (*b*) with a good spring and smooth but very sharp points. These forceps will enable the operator to catch hold of the cover-glass even when it is lying on a perfectly smooth surface. The lower cover is seized with the clamp forceps, fixed and held in the left hand. The second glass is taken up in the forceps (*b*) with the right hand, and applied to the drop of blood issuing from the prick in the finger. The cover-glass must pick up the blood without touching the finger itself. Next the cover-glass in the right-hand pair of forceps is carried rapidly to the other and allowed to fall gently on it. The blood then distributes itself in an absolutely uniform capillary layer, without the application of any pressure, provided that the cover-glasses are suitable. The upper

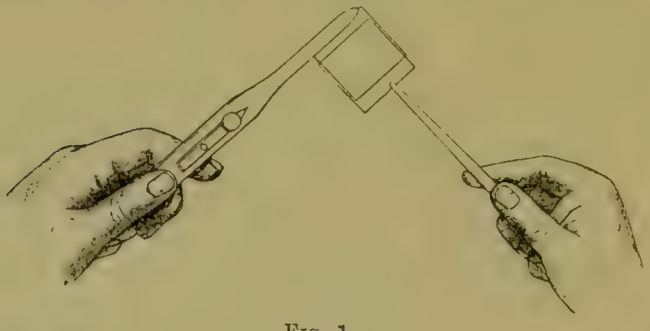

Fig. 1.

[1] Klönne and Müller, Berlin, make the forceps, as devised by Ehrlich.

glass is then seized, either with two fingers of the right hand, or better still with the forceps (*b*), and is carefully slid off the lower cover-glass, which is still held by the clamped forceps, but without pressing or raising it (see Fig. 1). As a rule only the lower glass yields a perfectly uniform smear, but at times both are utilisable. During the process of drying in the air, which takes about ten to thirty seconds, it is of course necessary to protect the cover-glasses from moisture, such as that derived from the breath of persons in the vicinity.

The size of the film on the cover-glass depends on the size of the drop which has been picked up. The smaller this has been, the smaller will be the surface over which the blood will distribute itself. Large drops are absolutely useless, if they cause the one cover-glass to swim on the other instead of merely sticking them together.

The directions for this method may appear to be somewhat complicated, but a little practice will show that the technique can be easily acquired. The details have been described minutely, because the author frequently sees specimens which he considers to be quite unsuited for the purpose, although they have been made by men who have given special attention to the study of hæmatology.[1]

Jancso and Rosenberger have published the details of another

[1] *Translator's Note.*—A considerable number of English investigators dispense with the use of cover-glasses altogether. Slides of the best quality are employed and cleaned with utmost care. Boiling in sulphuric acid or nitric acid, subsequent rinsing in distilled water, washing in alcohol and storing in mixtures of alcohol and aether yield good results. Wright advises polishing with very fine emery cloth, stretched on a wooden block before use. The drop of blood is applied to the slide, and is spread over the whole length by means of a second slide. Before this is done it is necessary to select a second slide, which "dances," *i.e.* which shows a just perceptible concavity of one of its ends. The concave surface is then gently and lightly pushed up the slide bearing the drop of blood until the blood runs toward the edge by capillary attraction. The slide is then gently drawn downwards, when it will be found that the blood follows it. Under no circumstances may the slide be drawn over the blood. Wright performs this drawing in short regular jerks, so that the smear appears as a series of thicker and thinner transverse lines of blood right down the lower slide. Some workers have also used a thread of silk for the purpose of drawing the blood down the slide.

The advantage of dispensing with the cover-glass is obvious. In the first place, it is difficult to secure cover-glasses which are reliable; next, they are much more

method of making fixed blood smears. This method has been employed by a number of investigators. The drop of blood is picked up on the edge of one cover-glass from the finger, and the edge of this cover-glass is then drawn gently over the full length of a second cover-glass. With practice very nice thin smears can be obtained in this way, which dry rapidly in the air.

After the specimens have dried thoroughly they may be stored between layers of filter paper in glass vessels provided with properly fitting stoppers. For important cases, when it is desirable to preserve the smears for a considerable time, it may be wise to protect the smear from the damaging effect of the atmospheric air by covering it with a layer of hard paraffin. Before the films can be fixed and stained it is then necessary to remove the wax by dissolving it in toluol or xylol. It is, of course, essential to keep the films in the dark.

The procedure may be modified in a number of ways according to the object of the examination. For example, for the detection of blood parasites it is advisable, according to Robert Koch, to use very large drops of blood. The blood cells, which are of minor importance in these cases, may be dissolved, and the detection of the parasites may be rendered much more easy by the use of a considerable quantity of blood.

(*b*) Fixing a Dry Specimen.

All methods of staining blood cells require a preliminary fixation of the albuminous substances in the blood. General directions for fixation cannot be given, since the intensity of the same must depend on the choice of the method of staining.

difficult to clean than slides, and are at the best of times liable to break. Further, the slide yields a much larger surface for examination, which may be of great importance, especially when it is difficult or impossible to secure another specimen of blood. The slides keep well, and are examined without any cover-glass, the cedar-wood oil being applied directly to the film and the objective immersed in the oil. The oil can be removed from the smear without damaging the latter by means of xylol or other solvent, if applied with care. This should be done after the examination is completed, and not left until a future occasion.

Comparatively small degrees of hardening suffice when the staining is carried out with watery solutions, such as the triacid solution. This may be achieved by allowing various agents to act for a short time, and in not too intense a manner. Other methods, which include the use of strong acids or which are carried out with solutions containing free alkalies, require that the structure should be fixed by a much stronger action of the fixatives. It is just as important to avoid over as under fixation. It is quite easy to determine the optimum fixation for each of the few staining solutions which are in use.

The following means of fixation may be employed:—

1. DRY HEAT.

For this purpose a simple copper plate placed on a stand is heated at one end by means of a Bunsen burner. After the flame has been burning for some little time it may be assumed that the plate has acquired a certain degree of constancy of temperature. It will, of course, be hottest at the burner end, and least hot at the other end. By allowing water, toluol, xylol, and other fluids to drop on the plate, the observer can easily ascertain which portion of the plate has approximately the temperature at which the various fluids boil.

Victor Meyer's apparatus, which is much used by chemists, is more suitable for this purpose. A modification adapted to the fixing of specimens takes the form of a small copper kettle, the cover of which is a thin copper plate having only one opening for the transmission of the steam pipe. If a small quantity of toluol is allowed to boil in the kettle for a few minutes, it may be assumed that the temperature of the copper lid is also between 107° and 110° C.

It is sufficient for specimens which are to be stained by the ordinary watery solutions to expose the cover-glasses to a temperature of about 110° for a half to two minutes. When differential staining (such as the eosin-aurantia-nigrosin mixture)

is to be employed, it may be necessary to expose them for longer periods or to higher temperatures.

2. CHEMICAL MEANS.

(*a*) Nikiforoff advised fixing the smears in mixtures of equal parts of absolute alcohol and aether for two hours, in order to obtain good triacid staining. Specimens fixed in this manner, however, are not so fine as those fixed by heat.

(*b*) Absolute alcohol fixes dry smears within five minutes sufficiently for subsequent staining by Chenzinsky's solution or by hæmatoxylin-eosin solution. In some cases, when it is desirable to examine the specimen quickly, it may be advisable to boil the dried cover-glass for one minute in a test tube with absolute alcohol.

(*c*) For Giemsa staining it is advisable to fix in absolute methyl alcohol. If this is undertaken immediately after the drying the fixation takes from three to five minutes, while if it is carried out on the following day it only takes two minutes.

(*d*) Formol was first employed by Benario in 1 per cent. alcoholic solutions for the fixation of blood specimens. The fixing is completed in one minute, and may be used for the demonstration of granulations. Benario recommends it especially for staining with hæmatoxylin-eosin.

Schüffner obtained beautiful results by fixing his blood smears in 1 per cent. formol solution to which from 5 to 10 per cent. of glycerine had been added.

For certain forms of staining the fixation is carried out simultaneously with the staining (see below).

It must be understood that these methods are described as the most suitable for blood examination in general. For special purposes, *e.g.* the demonstration of mitosis, of blood platelets, etc., the fixation methods generally employed in other branches of histology may be used with advantage. These include perchloride of mercury, osmic acid, and Flemming's solution *inter alia*.

(*c*) Staining a Dry Specimen.

Staining methods may be classified according to the purpose for which they are used.

In the first place, stains are employed for the purpose of obtaining rapid information of a general character. This may be attained by solutions which stain both the hæmoglobin and the nuclei (hæmatoxylin-eosin, hæmatoxylin-orange).

In the next place, it is at times desirable to have a staining which only affects one special form of cell in a characteristic manner, such as the eosinophile cells, the mast cells, or bacteria. This is termed "single staining," and is carried out in accordance with the principle of maximal decolorisation (see. E. Westphal).

In the last place, there is the so-called *panoptic* staining, *i.e.* staining which affects as many elements as possible, and which makes use of the greatest variety of colours. These methods are naturally of considerable interest for exhaustive examinations. It is necessary when they have been employed to utilise high magnification for the study of the specimens, but apart from this fact the methods yield more information with regard to the condition of the blood than any other. In order to obtain the greatest possible degree of differentiation, it will be found that double staining is usually not sufficient, but that it is necessary to use three colours which contrast from one another as much as possible. Formerly, the various stains were applied successively for this purpose. But, as every one who has employed these methods knows, it is exceedingly difficult to obtain constant results in this way, for even when the directions with regard to the length of time of staining and the concentration of the solutions are followed with the most minute care, it is impossible to rely on the results.

On the other hand, the methods of simultaneous or combined staining offer undoubted technical advantages. Improvements in technique are of considerable importance for the development of the histology of the blood. Since it appears that there is some want of clearness in the mind of some observers with

regard to the principles, a short description of the theory of differential simultaneous staining may be of use.

For this purpose a simple example will be selected. This is the employment of picro-carmine, *i.e.* of a mixture of neutral ammonium-carmine and a salt of picric acid. If a tissue which is rich in protoplasm be stained with carmine, the stain appears to be fairly diffuse, even though the nuclei become prominent. But if picrate of ammonium of the same concentration be added to the solution, the staining gains greatly in definition, by some portions appearing pure yellow and others pure red. The best known example of this is the staining of muscle by picro-carmine. In this case the muscle substance is stained pure yellow, while the nuclei take on a red colour. Now, if, instead of adding the picrate of ammonium, the experimenter uses a dye containing more nitro groups, such as the ammonium salt of hexa-nitro-diphenylamin, the carmine staining will be prevented altogether. All the elements in this case take on the pure colour of aurantia, no matter how long the stain is allowed to act. The explanation is very simple. Myosin possesses a greater affinity for picrate of ammonium than for carmine, and therefore combines with the yellow dye contained in the mixture of both stains. This combination removes the possibility of it taking up any carmine. The nuclei, however, possess a greater affinity for carmine, and therefore stain red in this process. But if a nitro dye be added to the carmine solution, which possesses a greater chemical affinity for all the elements of the tissue, and even for the nuclei than the carmine itself, the sphere of action of the carmine will be more and more limited, until when a very strongly acting nitro stain—the hexanitro compound—is used, it will be prevented altogether. Connective tissue, bone substance, and similar tissues, however, behave in a different manner toward the picro-carmine mixture. In this case the diffuse staining is solely dependent on the concentration of the carmine, and is not influenced by the employment of a chemical antagonist. It therefore is only possible to obtain a limitation of this staining by diluting the stain, and no addition of a dye stuff possessed of opposite

characters will make any difference. This example of tissue staining may be regarded as a mechanical attraction of the colour by the tissues, and not as a chemical combination. It may therefore be stated that a chemical staining may be recognised by the fact that it reacts to chemical antagonists, and that a mechanical staining reacts to physical modifications. This statement, however, is true only as long as pure neutral solutions of stains are employed. All additions, such as those of acids and alkalies, which could alter the chemical behaviour of the tissue, or which could diminish or increase the affinity of the tissues to the dyes, would also interfere with this test. From this view it may be deduced that all double staining methods, which can be employed by means of successive staining, can be advantageously substituted by combination staining, provided that it can be proved that the staining depends on a chemical combination. And, conversely, all those methods of double staining which can only be obtained by successive staining must be dependent on mechanical processes.

Only pure chemical staining processes are employed for the purpose of staining dry blood films, and the application of polychromatic combination staining is therefore possible in all cases.

The following combinations are available for blood preparations :—

1. **Combination Staining with Acid Dyes.**—The best-known example of this is the eosin-aurantia-nigrosin mixture, with which the hæmoglobin stains orange, the nuclei black, and the acidophile granules red.

2. **Mixtures of Basic Dyes.**—It is a simple matter to prepare mixtures of two dye bases. The most suitable of these are fuchsin, methyl-green, methyl-violet, methylene-blue, and pyronin. On the other hand, it is somewhat difficult to form a mixture of three of these substances, and this can only be done successfully by paying minute attention to the quantitative relationships. The following may be employed for this purpose : —fuchsin, Bismarck-brown, chromic-green.

3. **Neutral Mixtures.** — These mixtures were first introduced by Ehrlich into use for the histology of the blood, and for general histology. They have been found to be of considerable importance and claim careful consideration.

Neutral staining depends on the fact that nearly all basic dyes (*i.e.* the salts of dye bases, *e.g.* rosanilin acetate) enter into combination with acid dyes (*i.e.* salts of dye acids, *e.g.* picrate of ammonium) to form what is known as neutral dyes (*e.g.* rosanilin picrate). Their use is, however, rendered difficult by the fact that they are very little soluble in water. It was only after Ehrlich had shown that certain series of the neutral dyes are freely soluble in the presence of an excess of acid dyes that their use was rendered practically possible; in this way stable solutions of varying concentration of these dyes can be prepared. The most suitable basic dyes for this purpose are those which contain a so-called ammonium group, and especially methyl-green, methylene-blue, amethyst-violet[1] (tetra-ethyl-safranin-chloride), and, under certain conditions, pyronin and rhodamin. In contradistinction to these, those dyes which form the members of the triphenyl-methane series are on the whole but little suitable for this purpose, with the exception of methyl-green. These include fuchsin, methyl-violet, Bismarck-brown, phosphin, and indacine. The most suitable acid dyes for the purpose of forming the neutral dyes are more especially those highly soluble salts of the polysulphonic acids. The salts of the carboxylic acids and the other phenol dyes are less suitable, while the nitro dyes are the least suitable. The following members of the acid dyes may be enumerated, as being used for the purpose of forming neutral dyes: orange G, acid fuchsin, narcein (a freely soluble yellow dye, the sodium sulphanilate of hydrazo β-naphthol).

If a solution of an acid dye, such as orange G, be dropped slowly into a solution of methyl-green a coarse precipitate at first takes place, which is completely redissolved on the addition of more orange G solution. The solution is prepared so that the

[1] Kalle & Co., "Badische Anilin und Sodafabrik."

quantity of orange G is just sufficient to dissolve all the precipitate. A solution prepared in this manner is a typical example of a simple neutral dye solution. The example given may be explained chemically. All the three basic groups of the methyl-green in this mixture are combined with the acid dye, so that a triacid compound of methyl-green results.

Simple neutral mixtures which have one component in common may be combined with one another without further difficulty. This is a very important fact for triple staining, which has proved itself of utmost value. It can only be achieved by mixing together two simple neutral mixtures, *i.e.* two mixtures which consist of two components each. Chemical dissociation does not take place under these conditions. The important group of staining mixtures containing three or more dyes are obtained in this way. Theoretically, there are two main possibilities for such combinations.

1. *Dye mixtures,* composed of one acid and two basic dyes. For example:—

Orange–amethyst–methyl-green.
Narcein–pyronin–methyl-green.
Narcein–pyronin–methylene-blue.

2. *Dye mixtures,* composed of two acid dyes and one basic dyes. For example:—

Orange G–acid fuchsin–methyl-green,
Narcein–acid fuchsin–methyl-green,

and also the corresponding combinations of methylene-blue and amethyst-violet. The first of these mixtures will be described in greater detail later.

The importance of these neutral dye solutions depends on the fact that the mixtures colour certain structures separately which cannot be demonstrated by any of the constituents alone, and which are therefore called *neutrophile.*

Elements which, as is the case with the nuclein substances, possess an affinity to the neutral dyes take on the colour of the

basic dyes from such neutral mixtures; while the acidophile elements are coloured by one of the two acid dyes of the mixture. Those portions of the tissues which, owing to the presence of definite groups, have equal affinity to the acid and basic dyes, attract the combined neutral dyes to themselves, and are therefore stained the colour of the mixture.

Among the many dye combinations which microscopists, and especially hæmatologists have tried, the mixtures of **methylene-blue and eosin** have attracted especial attention. The extraordinarily beautiful colour contrast between these two substances which is not likely to occur frequently is to a large extent responsible for this. Not only have the details of a large number of very active and handy methods of staining with methylene-blue and eosin in two stages been published, and some of these have been found to yield good results in practice, but several dozen formulæ for the preparation of eosin-methylene-blue mixtures for simultaneous staining have also been described. It is quite impossible to enumerate all these mixtures and methods in this place, and it is presumed that it will be found impossible for any one student to test the adequacy of each of them thoroughly. Mention will therefore only be made of the more important of these, and of those which have proved themselves in the hands of the authors to be specially efficient.

Three different groups of these mixtures can be described. The first of these includes those formulæ which have aimed at the preparation of the most favourable proportions in the mixture of the two dyes, without any attention having been given to the reciprocal chemical action when applied to the specimen (*e.g.* Chenzinsky's solution); the second and third are based on a fact, which was first discovered by Romanowsky, that when mixed in certain proportions these two dyes in solution form a new chemical substance. This possesses in its nascent condition specific tinctorial characteristics which are not possessed by either of the dyes in their original solutions. In the course of his studies of the malaria parasite, Romanowsky

was able to obtain exceptionally good chromatin staining in this way, and less regularly good neutrophile granule staining. The discovery of the "red in methylene-blue," *i.e.* methylene-azure, was made from this observation, and it also led to the recognition of a stable "eosinate" of methylene-blue which demonstrates the neutrophile elements with certainty.

On repeating Romanowsky's method of chromatin staining, other observers failed to obtain regular results. Ziemann, however, was able to show how constant results could be obtained after he had tried all the commercial preparations of methylene-blue, and had further employed borax in addition. Nocht was the first to recognise that the active substance in this staining was a constituent of commercial methylene-blue, which he termed "red in methylene-blue." It was left to L. Michaelis, however, to explain this behaviour chemically, thereby materially advancing the practical solution of the difficulty. He was able to prove that active solutions of this kind, among which Unna's polychromic methylene-blue must be classified, contain methylene-azure beside unaltered methylene-blue. This substance has been described by Bernthsen, who regarded it as a sulphonic dye; but Kehrmann was able to show that this was not the case, but that it was a simple dimethyl-thionin. These researches have rendered it easy to prepare the active dye synthetically, and it can now be obtained in a pure condition,—for example, from the "Badische Anilin- und Sodafabrik." The most advanced use of this methylene-azure staining for hæmatological purposes takes the shape of "Giemsa" staining, which is justly much in vogue at the present time (see below).

A number of investigators, including Rosin and Michaelis, have contributed towards the gaining of a constant eosinate of methylene-blue, on which the hæmatologist can depend. It must, however, be mentioned that Jenner described, as long ago as in 1899, the most perfectly active product. This compound was obtained by an extremely simple method. His stain yields a true panoptic staining; the oxyphilic, basophilic, and neutrophilic elements of the normal blood are characterised sufficiently sharply,

and all the most important pathological changes are demonstrated by its means. The differentiation of the neutrophile from the eosinophile granules may not be quite sharp enough for an inexperienced observer, and in this case it might appear that a control with triacid staining would be advantageous.

In this way scientific research has given the practitioner a number of methods which, while they can be applied with absolute ease, guarantee the results with precision. These methods have been arrived at as the result of endless ingenuity and diligence, by means of which great difficulties have been overcome.

For practical use, apart from the solution of iodine and iodine eosin (which will be described on pp. 52, 53), the following claim attention :—

1. **Hæmatoxylin Solutions with Eosin or Orange G**—

R.	Eosin (cryst.) . . .	0·5 grm.
	Hæmatoxylin . . .	2 „
	Alcohol abs.	
	Aquæ dest.	
	Glycerini . . .	āā 100 grm.
	Acid acetic glac. . .	10 „
	Alum in excess.	

The solution must ripen for some weeks. Films fixed by a short exposure to heat, or in absolute alcohol, stain in from a half to two hours. The hæmoglobin and the acidophile granules stain red, while the nuclei take on the colour of the hæmatoxylin. The stain must be rinsed off very carefully.

2. For the practical employment of the **triacid solution**, it is especially necessary that the stains employed should be chemically pure. Heidenhain first pointed this out.[1]

The advantage of solutions made with such dyes is particularly well exemplified by the following observation. Formerly what was regarded to be basophile granules were

[1] At M. Heidenhain's request the "Aktiengesellschaft für Anilinfarbstoffe," Berlin, have prepared the three dyes in a crystalline condition.

frequently seen in white blood corpuscles, especially in the neighbourhood of the nucleus. Even such an experienced observer as Neusser did not regard these granules as artifices. They were therefore described as perinuclear structures, and were looked upon as being true cellular elements. Since the introduction of pure solutions of the dyes, these so-called basophile granules are not often seen.

Saturated aqueous solutions of the three dyes are first made up and allowed to stand until clear. They are then mixed in the following proportions:—

13–14	c.c.	of orange G solution.
6–7	,,	acid fuchsin solution.
15	,,	distilled water.
15	,,	alcohol.
12·5	,,	methyl-green solution.
10	,,	alcohol.
10	,,	glycerine.

These ingredients must be measured out in the same measure glass and added in the order given. After the methyl-green has been added the mixture must be well shaken up. The solution is ready for use at once, and will keep for a long time. Staining blood films with triacid need only be preceded by slight fixation. The staining itself is complete within five minutes.

The nuclei then appear greenish the red blood corpuscles orange, the acidophile granules copper colour, and the neutrophile granules violet. Mast cells are seen as peculiarly pale, almost colourless cells, with pale green nuclear substance. This behaviour is spoken of as negative staining.

It will thus be seen that triacid staining is, technically speaking, quite simple. It can be recommended for the purposes of gaining a general conception of the changes in a given specimen, and must be regarded as being indispensable in all cases in which the neutrophile granules have to be studied.

3. **Double basic Staining.**—A saturated aqueous solution

of methyl-green is mixed with a small quantity of an alcoholic solution of fuchsin.

The fixation for this method need only be slight, and the staining itself is complete within a few minutes. The nuclei appear green, the red corpuscles red and the protoplasm of the lymphocytes take on the colour of fuchsin. This method is therefore particularly suitable for films demonstrating the changes in lymphatic leukæmia.

3*a*. **Pappenheim's double basic staining with Pyronin Methyl-green.**—The directions, according to Grawitz, are as follows. The two following solutions are prepared :—

1.	Acid carbol. liq. . .	0·25
	Aquæ dest. . . .	100
	Methyl-green (pure) . . .	1·0
2.	Acid carbol. liq. . .	0·25
	Aquæ dest. . . .	100·0
	Pyronin	1·0

15 parts of No. 1 are mixed with 35 parts of No. 2, shaken up, filtered, and allowed to stain for a few seconds. The fixation must be carried out with heat. The solution can be obtained ready for use from Grübler.

4. **Eosin-methylene-blue Mixtures.**

(*a*) *Chenzinsky's solution.*

Concent. watery solution of methylene-blue . .	40 c.c.
Half per cent. solution of eosin in 70 per cent. alcohol	20 ,,
Distilled water	40 ,,

The solution is fairly stable, but should nevertheless always be filtered before use. The films need only be fixed by immersion in alcohol for five minutes. The staining takes from six to twenty-four hours, and is carried out in the incubator in air-tight block glass pots.

The nuclei and the mast cell granules stain an intense blue, malaria plasmodia stain a delicate sky-blue, the red blood

corpuscles and the eosinophile granules take on a beautiful red colour.

This solution is therefore especially suitable for the study of nuclear structure and of basophile and eosinophile granulation. It is largely used for anæmic and lymphatic leukæmic blood.

(*b*) *Von Müllern's Successive Staining.*—This method has been described as follows by Türk, who recommends it warmly:—

(*a*) Pure French eosin, ½ per cent. in 70 per cent. alcohol.

(*b*) Methylene-blue (B. pat.) ¼ per cent. in water.

1. Fixation for three minutes in methyl-alcohol.

2. The films are transferred directly to the eosin solution, in which they stain for from three to five minutes.

3. They are then rinsed with distilled water and dried between layers of blotting-paper.

4. They are then placed in a mixture of 20 drops of the methylene-blue solution and 10 drops of the eosin solution for from a half to at most one minute. The proportions for this mixture must be exactly measured, and it must be prepared fresh for each staining.

5. Rapid rinsing with distilled water, and rapid drying between layers of blotting-paper. Mounting in Canada balsam.

(*c*) Ten c.c. of a 1 per cent. aqueous solution of *eosin*, 8 c.c. of methylal, and 10 c.c. of a saturated aqueous solution of *methylene-blue* (medicinal) are mixed together and used at once. The staining is continued for one or at most two minutes. The staining is only characteristic if the films have been thoroughly fixed by heat. The mast cell granulations are coloured pure blue, the eosinophile granules red, and the neutrophile granules the same colour as the mixture.

(*d*) *Ziemann's solution*, which is specially adapted for malaria specimens and for the demonstration of lymph cells.

(*a*) One part of Hochst's medical methylene-blue in 100 of distilled water and 2 to 4 parts of borax.

(*b*) Höchst's eosin A. G., 0·1 per cent. in watery solution.

These solutions are mixed in proportion of 1 : 4. The film is

fixed in alcohol and is stained for five minutes. A metallic skim which forms on the solution should be removed with blotting paper, to prevent it from coming into contact with the film. The film is then rinsed well in water, immersed several times in very dilute acetic acid, and dried.

(*e*) In the next place, there are the solutions which actually contain "eosinate of methylene-blue" (Jenner, May-Grünwald). Methyl-alcohol is employed as the solvent, so that the fixation and staining take place at the same time. The authors recommend the hæmatologist to obtain either the eosinate of methylene-blue or the solution ready for use directly from Grübler of Leipzig. Burroughs, Wellcome & Co. put up the dye in small so-called "soloids."

A half to 1 per cent. solution of eosinate of methylene-blue in methyl-alcohol is prepared, and the films, after having dried in the air, but without any previous fixation, are immersed in the solution for about five minutes. They are then thoroughly rinsed off with distilled water, during which process they are decolorised to a certain extent. They are then dried and mounted in Canada balsam.

When stained in this manner, the red blood corpuscles and the eosinophile granules appear bright red, the neutrophile granules a paler red, the nuclei and the mast cell granules blue. The cytoplasm of the malaria plasmodium also appears pale blue. Granulated erythrocytes can be demonstrated well by this method.

(*f*) *Giemsa's Staining.*—The preparation of methylene-azure solutions is still very complicated, and the results in the hands of inexperienced workers are uncertain. It is therefore wiser to buy Giemsa's stain solution ready for use from Grübler of Leipzig, or Klönne & Müller of Berlin.

The fixation is performed for ten to twenty minutes in absolute alcohol, or from two to five minutes in methyl-alcohol. For staining blood films, 1 drop of Giemsa's stain is added to 1 c.c. of distilled water.[1] This is allowed to act for from ten

[1] The translator prefers a stronger solution of Giemsa for blood films (2 drops per c.c.) to act for three to six minutes. For parasites a weaker solution is, however, preferable.

to thirty minutes. The films are then rinsed with distilled water and dried. The erythrocytes appear pale red, the nuclei of the mononuclear and polynuclear leucocytes bright red and violet respectively, parasites and the plasma of the lymphocytes, blue, neutrophile granules violet-red, the acidophile granules a brownish-red, the mast cell granules a mauve colour, and the granulation of the erythrocytes blue or at times red.

What is termed "vital staining" requires to be dealt with separately. This term is a very unsuitable one. Ehrlich first employed it for the staining of nerve tissue in the living animal, and in this connection it is descriptive, since it actually conveys a correct meaning. But blood leaves off being a living tissue when it leaves the body, and begins to die from the moment it is abstracted from the vessels, even though under favourable conditions it is possible to retain the form of the elements unaltered for a surprisingly long time. It must, however, be admitted that some of the authors who employed the term (*e.g.* Rosin and Bibergeil) realised that it was not a satisfactory one. It would, however, have been wiser if they had avoided its use altogether and substituted for it such a term as "post-vital" or "prae-mortal" staining. But whatever name is given to this method, the author is of opinion that it has not served any purpose which could not be served in a much more convenient manner by stained dry films. It is necessary to accept with the utmost caution and reserve all that is claimed as new, since all sorts of uncontrollable phenomena may be produced in the process of dyeing when this staining is employed. It must even awaken suspicion, if this method of examination reveals appearances which cannot be demonstrated in dry stained preparations. These remarks, of course, are not intended to apply to the study of the phenomena of movement of the blood, which cannot be achieved in any other manner. The methods of vital staining depend either on the solution of the still fluid blood in the solution of the dyes (Arnold, Pappenheim), or on the principle

of applying the blood to cover-glasses on which an alcoholic solution of the dyes has been previously allowed to dry (Nakanischi, Unna, Rosin, and Bibergeil); or, lastly, on the principle elaborated by Deetjens, in which the examination is undertaken in a medium on which the blood can be preserved for a considerable time, and in which the dyes have been impregnated (A. Wolff).

Two further important methods for which dry blood films are utilised without any previous fixation must be briefly described before this subject is left and the subject of the histology of the blood proper is entered upon. These are: (1) The detection of glycogen in the blood, and (2) the microscopical demonstration of the distribution of alkali in the blood. To these may be added (3) the so-called diabetes reaction.

1. Recognition of Glycogen in the Blood.

This can be carried out in two ways. The original method consisted in placing the blood in a drop of a thick, cleared solution of iodine rubber under the microscope, in accordance with the glycogen test devised some time ago by Ehrlich. The following method, however, is a better one. The blood is placed in a closed vessel containing iodine crystals. Within a few minutes it takes on a dark brown colour. It is then embedded in a saturated solution of lævulose, which, as is well known, has a very high refraction index. It is necessary, in order to preserve such specimens, to use a cover-glass cement.

When either of these methods has been applied, the red corpuscles, having taken on the iodine stain, become prominent, without showing any morphological changes. The white cells are only stained to a slight extent. But all the structures which contain glycogen, be they in the white blood corpuscle, be they in the blood platelets or extracellularly in the débris, appear very markedly characterised by a beautiful mahogany-brown colour. The second modification of the method possesses this advantage, that on account of the strikingly refractive action of

the lævulose syrup the colour can be seen very clearly, while with the iodised rubber solution small quantities of glycogen in the cells may be obscured, partly by the opaque nature of the rubber and partly by the colour of the solution itself. The second, sharper method may therefore be recommended for more extended use in the examination of diabetes cases and of other diseases.[1]

The result is slightly different if the blood smear is exposed to iodine vapour while still moist (Zollikofer). When treated in this manner normal white blood corpuscles are found to be impregnated by a large number of granules stained deep brown, while the erythrocytes are stained much more intensely than by the dry method.

2. The Microscopical Test for the Distribution of Alkali in Blood.

This method is based on the test for alkali in glass, which Mylius has worked out. Iodide of eosin is a red compound which is very soluble in water but insoluble in aether, chloroform, and toluol. On the other hand, the free acid of the dye, as precipitated from the salt by acidifying the solution, is very little soluble in water, but is readily soluble in the organic solvents. On shaking it up thoroughly in an aethereal solvent the precipitate forms a yellow solution. If such a solution is applied to the surface of glass, on which alkali has been deposited as a result of the decomposition of the glass, the deposit takes on a brilliant red coloration, as a result of the formation of the highly coloured salt compound.

In applying this method to blood it is of course necessary to remove any deposition of alkaline salt from the vessels or cover-slips employed in the process by means of acid. The freshly prepared dry film is thrown into a glass vessel containing a solution of free acid iodide of eosin in chloroform, or chloroform

[1] This method can be warmly recommended also for the detection of glycogen in the secretions, *e.g.* in gonorrhœal pus, which always shows a marked glycogen reaction of the cells; the same reaction is further found in cells derived from tumours, either free in exudations or obtained directly from the growths themselves.

and toluol. The film soon becomes dark red in this solution. It is then rapidly transferred to another vessel containing pure chloroform, and the chloroform is changed once. The film is then mounted while still wet in Canada balsam. The morphotic elements in the film will be found to be well preserved. The plasma takes on a distinctly red colour, while the red blood corpuscles do not take up any stain. The white corpuscles show a red staining of the protoplasm, in which the nucleus, being unstained, appears as a vacuole (negative nuclear staining). The débris show intense red coloration, as does also any fibrin which may have been formed. These stainings are extremely instructive, and frequently demonstrate minutiæ which are not visible in preparations made by other methods, which aim more at obtaining elegant specimens. The study of these specimens is of the utmost value, because they show up all the artificial appearances and technical mistakes in a most reliable manner, and thus act as a sort of control to technique. The scientific value of the method consists in giving information with regard to the distribution of the alkali in the various elements of the blood. It appears that free alkali which reacts to iodide of eosin is not present in the nuclei; these structures must therefore possess a neutral or acid reaction. On the other hand, the protoplasm of the leucocytes is always alkaline, and it is found that the protoplasm of the lymphocytes contains the greatest amount of alkali. It is also necessary to call attention to the marked alkalinity of the blood platelets.

3. Bremer's Diabetes Reaction.

Bremer described in 1894 a peculiar colour reaction of the blood of diabetics. At first he employed a somewhat complicated procedure, but later described the following method. A fairly thick smear of the blood to be tested is made on a cover-slip, and this is fixed at 125° C. and stained at once in a 2 per cent. solution of methylene-blue. Diabetic blood when examined under the microscope is found to be stained a yellowish-

green, while normal blood takes on the colour of the stain employed.

Williamson's test may also be mentioned: 20 c.c. of blood (best taken by means of the pipette of Gower's hæmoglobinometer), 1 c.c. of a 1 in 6000 aqueous solution of methylene-blue, and 40 c.c. of a 6 per cent. solution of caustic potash are mixed in a test tube and boiled in the water bath for three or four minutes. Diabetic blood is either decolorised or tinted faintly yellow in this test, while normal blood solutions retain their colour. The test, as will be seen, is nothing more or less than a simple reduction test.

These facts have been confirmed generally by a number of observers (J. Loewy, le Goff, Hartwig, Dammer, and others), but at the same time it was found that the reaction is not specific for diabetes, but occurs at times in other conditions. There are considerable differences of opinion with regard to the interpretation of the phenomenon, and at present it is not possible to consider the matter settled. There is no doubt that the reaction in the blood of diabetics is produced by the presence of grape sugar. Loewy has proved that in Bremer's test the blood corpuscles cause the decolorisation, while in Williamson's test it is the plasma.

It is interesting to note that both Williamson and Loewy have still obtained the reaction in diabetics, after the glycosuria has disappeared.

According to Ehrlich, these phenomena may be explained on the assumption that a chemical combination between the glucose and some other constituent of the blood, probably the hæmoglobin, diminishes the power of taking up the methylene-blue. This view is supported by the fact that normal blood which has been treated with grape sugar gives a Bremer's reaction.

There is no doubt that this reaction occurs in other diseases in which there is no increase of the sugar of the blood.

B.—NORMAL AND PATHOLOGICAL HISTOLOGY OF THE BLOOD.

The Red Blood Corpuscles.

During the past ten years new views have been put forward with regard to the structure of the red blood corpuscles, which to a certain extent stand at variance with the old generally accepted ideas. Special mention must be made in this connection of the investigations of Weidenreich on the shape of the erythrocytes. As a result of extensive comparative anatomical studies he comes to the conclusion that the red blood corpuscles are not discs provided with symmetrical delled concavities on both sides, which when viewed in transverse section would show the so-called dumb-bell shape. He believes that they have the shape of a bowl or bell glass, or, as he very expressively explains it, are like a rubber ball from which some of the air has escaped and in which a dent has been made.

These views with regard to the shape of the red cells are of purely academic interest, and do not convey any influence on physiology or pathology. The various hypotheses with regard to the structure may be of importance. The older views of oikoïd and zooïd (Brücke), of discoplasm (Ehrlich), of a stroma in the meshes of which the hæmoglobin is distributed (Rollet, Hayem), were to a great extent based on the demonstration of a framework in the protoplasm of the cells by Arnold and his pupils, E. Bloch, Rosin, Bibergeil, and others. Weidenreich and Grawitz, however, contend that the protoplasm of the erythrocytes has a perfectly homogeneous structure. The latter bases his opinion on the experiments which he carried out with Grüneberg with ultra-violet rays. The observers named, as well as Hamburger and others, recognise a membrane which forms an external limit of the blood discs. Weidenreich believes that this membrane possesses basophile characteristics, and that it plays a part in the development of punctation and of blood platelets.

The existence of a membrane has been accepted by Köppe,

and by Albrecht and Hedinger, as the result of further methods of examination. The membrane belongs to the lipoid substances. It contains lecithin and cholesterine, and can be dissolved by definite physical and chemical means. These views, as will be shown subsequently, are of great importance for the doctrine of the pathogenesis of various forms of anæmia.

The nucleoid theory opposes the doctrine of the homogeneity of the red blood corpuscles. This theory assumes the remains of nuclear substance in the interior of the blood discs, which it claims to demonstrate by special forms of. staining. It has been supported by Pappenheim, Arnold, Hirschfeld, Maximow, and others, and has received remarkable confirmation by Löwit's observations with fresh blood specimens (quoted by Pappenheim), and by the dark-field illumination studies of Pappenheim. The latter describes the nucleoid substance as "molecularly motile," and as of "tenacious fluid consistence."

The views with regard to the red blood corpuscles which are of the greatest importance for clinical medicine, however, are not based on these special anatomical and physiological examinations, but have been formed as the result of the clinical methods described above, and especially those dealing with dry films.

A technically well made dry film shows the red blood corpuscles in their natural size and shape, and the cupping or delling can be distinctly seen. They present the form of isolated, round, homogeneous structures of a diameter of about 7·5 to 8·5 μ (maximum 9·0 μ, minimum 6·5 μ). They show the most intense staining in the peripheral zone, and the least in the dell or centre. The stroma does not take up any of the stains mentioned above. The dyes only affect the hæmoglobin, so that an experienced observer can gain some idea of the hæmoglobin content of the individual cell by the intensity of the staining, which is much more reliable than the natural colour of the hæmoglobin in fresh unstained preparations. Blood corpuscles which are poor in hæmoglobin are readily recognised by their pale staining, and especially by the lightness of the central zone. When this is very marked they appear as structures which

Litten has appropriately called "pessary" forms, on account of the fact that only the periphery stains at all. The slight power of staining cannot be explained, as Grawitz believes, by a smaller affinity of the hæmoglobin to the dye. Qualitative alterations of the hæmoglobin, which would modify its behaviour toward the dye, do not exist, even in anæmic blood. The fact that anæmic blood stains more faintly than normal blood depends entirely on its smaller hæmoglobin content.

A diminution of the hæmoglobin content may be ascertained in this manner in all the anæmic conditions, and especially in post-hæmorrhagic, secondary, and chlorotic anæmias. In contrast to this, however, as Laache first pointed out, there is an increase in the amount of hæmoglobin in a large number of erythrocytes in pernicious anæmia.

In order to form a correct conception of pathological changes in blood, it is necessary to bear in mind that the individual red blood corpuscles are by no means equal even in normal blood. Under physiological conditions some of the cells are constantly being used up and replaced by new ones. Each drop of blood therefore contains erythrocytes of varying age. It will be readily understood that damaging influences, provided that they are not too great, need not affect all the red blood corpuscles equally. The least resistant of the elements, *i.e.* the oldest cells, will be destroyed by these damaging influences, while the more robust only react in a more purposeful manner toward the same agencies.

The anæmic composition of blood as such undoubtedly constitutes a moderately intense stimulus of this kind. The action may be studied with advantage in cases of acute post-hæmorrhagic anæmia.

Certain characteristic changes are observed in the blood discs in all anæmic conditions.

A. **Polychromatophilia.**—This term is employed for a condition, which was first described by Ehrlich, in which the red blood corpuscles show a modification in staining, consisting in taking on a mixed colour, instead of a pure hæmoglobin tint,

as in normal blood. For example, the red blood corpuscles in normal blood films stained by the hæmatoxylin-eosin mixture are stained pure red. If a specimen of the blood from a case of chronic anæmia, in which all the degrees of degeneration are usually present, be stained in the same manner, it will be seen that some red cells have a faint trace of violet. The film will contain cells which have taken on a colour which at best may be described as a bluish red, and others, stained a fairly intensely blue, which do not show a trace of the red tint; these cells are frequently in such a condition, showing ragged edges, etc., that they may be regarded as dying elements.

Ehrlich has enunciated the theory, that this peculiar behaviour toward the stains is an indication of the gradual dying off of the red blood corpuscles, and especially of the older forms, and that this change leads to a coagulation necrosis of the discoplasm. The latter, as is always the case with coagulation necrosis, charges itself with the albuminous substances of the blood, and thereby acquires the power of taking up the nuclear dyes. At the same time, the discoplasm loses its power of retaining the hæmoglobin within itself, and consequently the pigment is discharged into the fluid portion of the blood in quantities proportional to the degree of the changes. Under these conditions the red discs show an increasing loss of specific hæmoglobin staining. Ehrlich speaks of this behaviour as "anæmic degeneration."

These views have been challenged by various observers—at first by Gabritschewski, and later by Askanazy, Dunin, etc. They contended that the polychromatophilic discs are not dying elements, but on the contrary are young forms. The chief fact on which this opinion was based was that in certain forms of anæmia the precursors of the nucleated red cells are frequently polychromatic, as far as their protoplasm is concerned.

In view of the great theoretical importance of this matter it is thought wise to briefly recount the reasons which have been put forward in favour of the degenerative character of the changes.

1. The appearance of those erythrocytes which show the highest degree of polychromatophilia. The raggedness of the edges lends them an appearance which everyone who is accustomed to study morphological changes would accept as an indication that a cell is undergoing solution and is markedly degenerate.

2. The fact that these changes can be produced to a marked degree in experiment animals, *e.g.* by inanition under conditions in which there can scarcely be any question of new formation of red blood corpuscles; and further the experiments of C. S. Engel, in which white mice, having been infected with hæmorrhagic septicæmia, show numerous polychromatic erythrocytes.

3. The clinical experience, that these staining anomalies may be seen in a large number of cells within the first twenty-four hours after an acute hæmorrhage in man. According to the author's extremely careful observations in this connection, involving many hundred cases, nucleated red blood cells are not found in man as early as this.[1]

As has been stated, a number of observers opposed the view which Ehrlich at first put forward of the degenerative nature of the phenomenon, and substituted for it the view that polychromatophilia is a peculiar characteristic of young elements.

This latter view has been supported by the investigations of Engel on the development of the red blood corpuscles. He showed that nucleated red blood corpuscles, both normoblasts and megaloblasts, frequently reveal, under perfectly physiological conditions, even a markedly polychromatic protoplasm. Naegeli has recently demonstrated that in early embryonal stages all the erythrocytes are polychromatic. Walker was able to show, in some comparative studies, that in certain classes of animals the circulating blood normally contains polychromatic red blood discs, while in other species this is not so. It therefore appears to be

[1] Dunin has described the appearance of nucleated red blood corpuscles within the first twenty-four hours after a hæmorrhage in man as a normal condition which may be observed regularly. The author, however, considers that this view is not in accordance with fact. He can only admit that single instances of such a rarity may be met with.

certain that polychromatophilia may also be a sign of young forms.

There is absolutely no reason why both views should not be accepted. There are other examples in histology, and especially in hæmatology, of young and dying elements having similar characteristics; mononuclear neutrophile cells must be regarded as the precursors of the polynuclear cells, and also without any doubt as a degeneration form of the same cells.

The genesis of this phenomenon is of course different for the instance when it represents a degeneration form to that when it represents an early developmental stage. In the former case it is usually dependent on acquired pathological characteristics of the circulating blood, produced by the influence of the surrounding blood plasma (see above). The staining peculiarities may also be produced, according to Engel, by the action of bacteria on the bone marrow.

With regard to the demonstration of polychromatophilia, suffice it to mention that Türk and Naegeli have found that methylene-blue alone, or Löffler's solution of methylene-blue, are to be recommended as the most suitable stains. Very beautiful results can be obtained with Chenzinsky's solution.

B. **The Punctated Erythrocytes.**—In various conditions, punctate and at times coarse deposits are met with in the protoplasm of the red blood corpuscles. These deposits cannot be seen in fresh specimens, but may be demonstrated in dry films by means of almost any of the nuclear stains. At times these deposits are so minute and so closely placed that the whole cell appears to be stained with the nuclear dye. For example, when stained with methylene-blue the cell offers an appearance of a homogeneous blue structure. In other cases the deposits are coarse, and look like little heaps of stain. In one and the same cell the deposit may be in part extremely fine and in part very coarse. Again, it may be distributed equally over the whole cell, or it may be limited to a part of it.

Such deposits were first found by Ehrlich in 1878, and mentioned in a dissertation, compiled under the supervision of

von Noorden. S. Askanazy described these elements minutely in a case of anæmia in 1893, and in 1894 Schaumann mentioned them in his monograph. A communication made by the author, in which he reported that he had found these elements in more than twenty cases of pernicious anæmia and in leukæmia, appears to have awakened general interest in these bodies. The author in his communication suggested the term of punctate erythrocytes for this condition of the red cells, since this term did not presuppose any special significance. It is essential to avoid the employment of any term which could suggest the slightest similarity between the punctate deposits and the granules of the leucocytes. Granules are structures which possess definite functions of considerable physiological and biological importance, while the punctiform deposits of the erythrocytes cannot be said to serve such a purpose. For this reason it would seem to be advisable to avoid using such designations as "basophile granulated erythrocytes," which might be easily confused with the description of mast cells by inexperienced observers.

An extraordinarily large number of communications followed the publication by the author, dealing with further characteristics of these bodies, while others discussed their whole significance (Klein, Zenoni, Lenoble, Litten, Borchardt, E. Bloch, Engel, Bourret, Sabrazés, Strauss and Rohnstein, A. Plehn, Grawitz and his pupils, P. Schmidt, Naegeli and his pupils, S. Askanazy and others).

These communications revealed the fact that punctate erythrocytes may be found in all forms of anæmia. It appears to be undoubted that this form of cell is especially marked in those anæmic conditions which either depend on toxic causes or in which these factors play a large part in the causation. Accordingly they are found almost invariably in all cases of progressive pernicious anæmia, carcinoma, etc. The extremely frequent occurrence in lead poisoning has awakened special interest, after Borchardt first called attention to the fact. It has, however, been proved that the phenomenon is not necessarily associated with toxic processes, since it has been seen repeatedly

in cases of pure post-hæmorrhagic anæmia, even if this is not the rule. This is not only true for hæmorrhages which take place into one of the body cavities, as Grawitz believed, under which conditions he concluded that a toxic action was produced by the absorption of the blood which had escaped from the vessels, but applies equally to those forms of hæmorrhage in which the bleeding takes place externally, and which are undoubtedly cases of pure acute post-hæmorrhagic anæmia (Bloch, Schmidt). Mention must be made of the following facts. The punctate erythrocytes have been found as the only ascertainable blood change in the stage of complete remission in progressive pernicious anæmia (Lazarus); their presence may form the first sign of lead poisoning, before any other symptoms have made their appearance; and lastly, according to Strauss and Rohnstein, they have been observed in a single instance, in a perfectly healthy young medical man, in which case the examination was carried out with extreme care.

Two questions have been thrown up by all the authors who have busied themselves with the subject, since these elements have been known to exist. Firstly, what is the general clinical significance of these structures; and, secondly, what is their histological explanation. The answers to these two questions naturally stand in close association to one another.

No one will dispute that the punctate elements are at all events in part derived from nuclear structures. This becomes quite evident when series of cells are examined, such as those depicted in Part II. of *Anæmia*, Plate 2, Fig. 5. In these cells an unbroken series of fragments from the largest portions of the nucleus, down to the very finest punctiform dust, is plainly visible. But this by no means proves that all such deposits are derived from nuclei. According to Grawitz, a certain difference in the staining speaks against the view that the deposits are derivatives of the nuclei. This objection, however, is just as little convincing as it would be if it were assumed that basophilic staining is direct evidence of a nucleogenous nature; fragments of nuclei, as Meyer and Speroni have appropriately pointed out,

need not behave chemically in the same way as intact nuclei. A further objection consists in the statement that this punctate deposit has never been discovered in bone marrow. Pappenheim, however, has shown that it is quite easy to demonstrate it in the medulla if the method of staining be but slightly modified. Schur and Loewy and Bloch have found it in the bone medulla. Naegeli was able to demonstrate the presence of punctate deposits in bone marrow, even in cases in which they were absent in the blood. In opposition to the assumption of the nuclear origin of the punctate deposits, Grawitz alleged that he had found innumerable punctate erythrocytes in the blood of many patients, without having come across a single erythroblast. Quite apart from the fact that this could not be accepted as evidence in favour of Grawitz's views, Meyer and Speroni have found, more especially in lead poisoning, that when the examination is undertaken regularly, erythroblasts are present in every case which shows punctate erythrocytes. The third argument against the view is that punctate deposits are found in erythroblasts with absolutely intact nuclei, and in cells which show mitosis. But it must be pointed out that it is not unreasonable to suppose that the punctate deposits could have been derived from the disintegration of a second nucleus, or that they may have been formed during the process of division (Schmidt).

Meyer and Speroni have recently called attention to a highly important fact. In animal experiment, punctiform deposits can only be produced in those warm-blooded animals whose red blood corpuscles normally are not nucleated. If the phenomenon depended on some damage to the protoplasm, it would be difficult to understand why it should be absent in those warm-blooded animals whose red blood corpuscles contain nuclei. The punctiform deposits only occur when denucleisation takes place as a result of dissociation of nuclei under physiological conditions.

It may be deduced from the foregoing that some of the basophile punctiform deposits undoubtedly owe their origin to the nuclei, while it cannot be proved that they stand in any

causal relation to the protoplasm of the cell. Under these circumstances it seems unnecessary to attempt to explain one morphological phenomenon in two ways.

Since the close relationship between the punctiform deposits and the nuclei suggests that this phenomenon is more of the nature of a regenerative than a degenerative process, it is interesting to note that further facts have been brought to light which support this view. The most striking evidence has been obtained by experiment. Sabrazès, and Naegeli and Lutoslawski, whose work fully confirms the work of the first named, have shown that punctate erythrocytes can be readily produced in chronic lead poisoning, but that they disappear as soon as the dose of poison has exceeded a certain point. This result can only be explained on the assumption that the punctate erythrocytes indicate that the bone medulla reacts actively, while when large doses are employed the medulla is paralysed. Analogous conditions are known to exist, *e.g.* in experimental arsenical poisoning (Bettmann). Schmidt has obtained results which tally well with this theory in his experiments with various other poisons.

As Naegeli has tritely pointed out, the appearance of this phenomenon in erythrocytes showing mitosis speaks in favour of it being a regenerative process. It would be absolutely inconsistent to suppose that a degenerative process could take place in the same cell at a time when the most energetic regenerative process is going on. Finally, it may be stated that the fact that basophilic punctiform deposits have been found in embryonal blood, that is, under conditions when all degenerative processes may be regarded as excluded, clinches the evidence in favour of this theory. This does not only occur in the embryonal blood of mice in which it was first observed by Engel, but, according to recent observation of Naegeli, it also takes place in the embryonal blood of all animals, including man.

In his last contribution S. Askanazy has admitted that these bodies are a sign of regeneration. But in contradiction to his earlier views he now considers that the punctiform deposits are

a variation of polychromasia, which he attributed to a peculiar abnormality of the plasma. His arguments are based on the alleged condition, which has been disproved in the preceding paragraph, that the punctate erythrocytes have never been found in bone marrow, and also on the assumption that this phenomenon appears side by side with polychromasia. This is certainly not the case as far as the individual erythrocytes are concerned. A large number of orthochromatic erythrocytes containing punctiform deposits are met with. And even if this assumption holds good for blood as a whole, it must be pointed out that this is not surprising, since the phenomenon of polychromasia is observed in nearly every case of anæmia.

In view of all these facts and theoretical considerations it appears that, although this may be to some extent at variance with the views expressed formerly by the author, the following conclusions are justified. The punctiform deposits are the derivatives of nuclear substance, and the process must be regarded biologically as a transformation of the nuclear substance, modified by pathological conditions, and perhaps as an altered form of denucleisation. The whole phenomenon therefore bears in its clinical significance the characters of a pathological regeneration.

Schleip found in a case of progressive pernicious anæmia that the blood, when stained by Leishman's and Giemsa's methods, contained in the normal or polychromatophilic erythrocytes, small rings or much twisted loops composed of extremely delicate threads. In the larger erythrocytes there were two or three rings, and at times rings were found lying free in the blood plasma, in which case remnants of a red blood corpuscle could be discerned adhering to them. Schleip was able to demonstrate these changes in a few further cases of progressive pernicious anæmia, in severe secondary anæmia, in chronic lead poisoning, in one case of acute leukæmia, and in one case of pseudo-leukæmia. He regarded these structures as remains of the nuclei, and possibly of nuclear membrane, and attributed the condition to an abnormally increased new formation. Schleip

has stated that Cabot described the same condition as early as 1903, and Gabriel confirmed Schleip's observation in a single case of progressive pernicious anæmia. Naegeli (private communication) has found very numerous ring bodies in a number of cases of infantile pseudo-leukæmic anæmia, and also in acute lymphatic and acute myeloid leukæmia, as well as in progressive pernicious anæmia (see Plate III.).

C.—A third change which is found in the red blood corpuscles in anæmia is known as **poikilocytosis** (Quincke). This term implies a change in the microscopical appearance of the blood, which is characterised by the presence of large, small, and minute red elements, in addition to a greater or smaller number of normal sized red blood corpuscles, "anisocytosis" (Strauss). As Laache first pointed out, and as has subsequently been confirmed by other hæmatologists, extremely large cells, which are rich in hæmoglobin, are found in Biermer's anæmia. In all other severe and moderately severe forms of anæmia the volume and hæmoglobin content of the red blood corpuscles are, as a rule, diminished. This apparent contradiction could not be explained by Laache, who first called attention to it, but has been satisfactorily accounted for by the results of Ehrlich's observations on the nucleated precursors of the megalocytes and normocytes (see below).

The appearance of anæmic blood becomes more complicated by the fact that the smaller cells do not retain their normal shape, but take on well-known irregular forms: pear shape, balloon shape, boat shape, and dumb-bell shape. At the same time, the central cupping can still be recognised even in the smallest forms in well-prepared dry films. The so-called microcytes form an exception to this rule. These are small globular bodies, which used in the early days of microscopical hæmatology to be regarded as being of prognostic importance in severe forms of anæmia. It has, however, been shown that these bodies are merely contraction forms of poikilocytes; or, in other words, the microcytes stand in the same relation to the poikilocytes as the crenated forms stand to the normal erythrocytes. Accordingly microcytes are

but rarely seen in dry films, whereas they may be seen in fresh specimens after prolonged examination.

It is further of importance to know that in fresh blood the poikilocytes show certain movements; this has given rise to wrong conceptions in many instances. They were regarded as the causal organisms of malaria in the early days of hæmatology; while the larger forms were looked on, at a later date, as amœbæ and similar organisms by Klebs and Perles. Hayem from the first described these forms as "pseudo-parasites," and warned the observer against assuming that they possessed a parasitic character.

Formerly the origin of poikilocytes was the subject of considerable discussion, but it is now generally accepted that the explanation which Ehrlich has given is the correct one. The fact that poikilocytosis can be produced in any specimen of blood by careful warming has led to the deduction that these forms are products of a fragmentation of the red blood corpuscles ("schistocytes," Ehrlich). This view is further supported by the fact that even the smallest fragments in dry films show a definite delling. They contain the specific protoplasm of the blood disc, the discoplasma, which "possesses the tendency of assuming the delled form in the stage of quiescence."

No other qualitative alterations of the protoplasm of the poikilocytes can be demonstrated even by staining. They may therefore be regarded as possessing full functions, and their presence may be ascribed to a purposeful reaction to counteract the diminution in the number of blood corpuscles. The respiratory surface is considerably increased by the disintegration of a large blood corpuscle into a number of homologous smaller cells.

D.—A fourth change in the morphological condition which is seen in blood in the severer degrees of anæmia is the presence of **nucleated red blood corpuscles.**

While the question of the origin of the elements of the blood cannot be followed up in this place in any degree of minuteness, it may be wise to describe in a few words the present position of the doctrine of the nucleated red blood corpuscles.

Nucleated cells have been generally accepted as the young forms of the normal red blood corpuscles ever since Neumann and Bizzozero published their standard works (October and November 1868). Hayem's theory, on the other hand, according to which the blood platelets are the origin of the erythrocytes, may be regarded as having been disputed by everyone save this author and his pupils.

Ehrlich called attention to the clinical significance of the nucleated red blood corpuscles in 1880, by pointing out that cells of normal size—normoblasts—occurred in the so-called secondary anæmias and in leukæmia, and very large elements—megaloblasts, gigantoblasts — occurred in Biermer's anæmia. Ehrlich at the same time emphasised the fact that the megaloblasts play an important part in the embryonal formation of blood. In 1883, Hayem sought to classify the nucleated red corpuscles into—(1) The "globules nuclées géantes," which were only to be found in the embryonal condition; and (2) the "globules nuclées de taille moyenne," which are present in the late stages of embryonal life and always in adults. W. H. Howell (1890) recognised two kinds of erythrocytes in cat embryos: (1) A very large blood cell, similar to the blood cells of reptiles and amphibia ("ancestor corpuscles"); and (2) a blood cell of the usual size of mammalian blood corpuscles.

Three forms of nucleated red blood corpuscles may be distinguished by the following characteristics:—

1. **Normoblasts.**—These are red blood corpuscles of the size of the ordinary non-nucleated discs; the protoplasm shows, as a rule, pure hæmoglobin coloration; they have one nucleus as a rule and at times there are from two to four. The nucleus is pycnotic, has well-defined edges, and apparently no differentiated structure. It is usually concentrically situated, and occupies the greater part of the cell. It is characterised by the capability of taking on a more intense degree of staining with the nuclear dyes than do the nuclei of the leucocytes, or indeed of all other cells. This property is so characteristic that, even when free nuclei are met with around which no or exceedingly little hæmoglobin can be

distinguished, as is the case at times in anæmias, and especially in leukæmia, they can be readily recognised as normoblast nuclei.

2. **Megaloblasts.**—These cells are from two to four times the size of normal red blood corpuscles. Their hæmoglobin occupies by far the greater part of the cell, and frequently shows more or less marked degrees of anæmic degeneration. The nucleus is larger than that of the normoblasts, but does not occupy so large a portion of the cell as the latter does. It is frequently ill defined, and has a rounded shape. The nuclei of the megaloblasts may be distinguished from those of the normoblasts by their finely differentiated structure and by their small capability of taking up nuclear stains, which may be so limited that an inexperienced observer may have difficulty in recognising that the cells have a nucleus at all.

At times cells corresponding to the form described above, but of a considerably larger size, are met with. These cells are termed gigantoblasts, but are regarded as being of the same type as megaloblasts.

3. **Microblasts.**—These forms occur at times, as, for example, in traumatic anæmias, but must be regarded as extremely rare cells. They have not attracted the especial attention of the hæmatologist up to the present.

Practically every hæmatologist of repute has followed Ehrlich's propositions in the morphological classification of the nucleated red blood corpuscles. Marked differences of opinion have only been expressed with regard to the significance of the two chief forms. There is no doubt that this difference of opinion depends in part on the fact that some of these investigators, in using the classification introduced by Ehrlich, have not adhered strictly to his definitions. It is not unreasonable to expect that when a name has been suggested for a definite matter, this name should not be employed to signify some other phenomenon or structure. This claim has not been respected in modern hæmatology. When an investigator considers that the suggested term is unsuitable in any respect, he can propose a new, better term, but he should not

create confusion by a false use of the original one. Many of the disagreements with regard to Ehrlich's doctrine concerning the normoblasts and megaloblasts would not have occurred if all the authors had respected this regulation. In order to gain a perfectly clear conception of the physiological and pathological import of the erythroblasts, it is absolutely necessary to consider, in the first place, only typical examples of both forms. There are, as would be expected, a large number of cells which cannot be classified either as normoblasts or as megaloblasts, on account of the fact that some of the characteristics which Ehrlich described for these cells are wanting. It would, however, be purposeless and would not contribute towards success if atypical intermediate forms were utilised for the purpose of gaining a clear insight into the significance of the definite and typical forms which can be accepted as standards.

Turning first to the physiological occurrence of normoblasts and megaloblasts, everyone is agreed that only the latter variety is met with in the earlier embryonal stages. This variety is gradually replaced by the former, so that the blood of the fully developed fœtus does not contain any megaloblasts, although the bone marrow still does so. The same remark applies at times to the first two years of life. No one has ever claimed to have found megaloblasts in the blood of healthy adults. In spite of very extensive observations, neither Ehrlich, Bloch, nor the author have ever found megaloblasts in the bone marrow of adults; while Dominici, Naegeli, Grawitz, Engel, and others claim to have seen megaloblasts as rare, and even very rare, constituents of normal bone marrow. These exceptional finds, however, suffice to impel Grawitz to regard the megaloblasts as "a type of normal blood formation." He seeks to establish this view by setting up a hypothesis of a development of megaloblasts as a result of an increased water content of the blood. While this assumption appears to be untenable in view of the function of the megaloblasts in the embryonal blood formation, and of the high hæmoglobin content of the cells, some recent experiments of Georgopulos have deprived it of

all foundation. This observer showed that in hydræmic conditions absolutely no swelling of the erythrocytes takes place, and he was even able to demonstrate that on the appearance of disturbance of compensation of the heart in persons suffering from renal affections, and from the consequent increase in the concentration of the blood, the diameter of the erythrocytes is increased on the average.

All other hæmatologists are agreed that the normoblasts of the bone marrow alone are the physiological precursors of normal red blood corpuscles. It is at present impossible to prophesy what explanation will be found for the contradictory observations of Ehrlich on the one hand, who failed to find megaloblasts in the marrow, and Dominici and others who found them on a few occasions.

It is necessary to consider the fate of the nuclei of the erythroblasts separately, in spite of the fact that this landmark is no longer regarded as important as it formerly was for the differentiation of the two forms of cells. For a long time two views with regard to the transformation of the nucleated erythroblasts into the non-nucleated erythrocytes were opposed to one another. Rindfleisch, who was the chief exponent of the one view, taught that the nucleus of the erythroblast issued from the cell, leaving it in the form of an erythrocyte, and that the nucleus itself took up, by means of a trace of protoplasm, which still adhered to it, fresh substances out of the plasma, imbibed hæmoglobin, and thus shaped itself into an erythroblast again. The other doctrine, which was directly irreconcilable to the former, assumed that the erythroblasts were transformed into non-nucleated discs by the disintegration of the nucleus within itself ("karyorrhexis, karyolysis"). Kölliker and E. Neumann may be particularly mentioned as having championed this view, and as having regarded it as the only way in which the erythrocytes are formed.

Rindfleisch arrived at his theory by direct observation of the phenomenon, which he described. He saw this take place in the blood of guinea-pig embryos and in teased bone marrow

preparations, both of which had been treated with physiological salt solution.

E. Neumann stated that Rindfleisch's doctrine was untenable, because the process which the latter had watched was simply the result of a lesion of the blood produced by the salt solution and by the teasing. When a method of preparing the specimens was selected, by means of which all chemical and mechanical changes in the blood could be avoided, the issuing of the nucleus did not take place.

Kölliker's and Neumann's view, that the nucleus gradually disintegrates in the interior of the cell, is not based on observation of the process, but is based on the fact that in suitable specimens, *e.g.* of fœtal bone marrow, blood of the liver, and also leukæmic blood, the transition from erythroblasts to erythrocytes can be demonstrated in every stage of nuclear metamorphosis; von Recklinghausen claims to have observed the solution of the nucleus within the cell itself in rabbit's blood, which was kept alive in the moist chamber. Pappenheim's criticism, that the last-named observer had watched a process analogous to that described by Maragliano and Castellino as artificial blood necrobiosis, should be mentioned in this connection.

Similar differences of opinion are displayed with regard to the significance of the "free" nuclei, which may be seen in many specimens. Kölliker taught that these nuclei were not absolutely free from protoplasm, but that a narrow investment of protoplasm could always be made out. Rindfleisch regarded them as nuclei which have been cast out of, or have issued from, the erythroblasts; while Neumann described them as young forms of erythroblasts.

The investigations of the last ten years have failed to reconcile the champions of these doctrines. It is unnecessary to discuss the methods and stages of examination which the various authors have followed. Suffice it to mention that the names of E. Albrecht, Howell, M. Heidenhain, v. d. Stricht, and Jünger have appeared in support of Rindfleisch; while Massloff and Naegeli have recently taken up the cudgels for Neumann and Kölliker.

Ehrlich first attempted to bridge over the gap placed between the two conflicting views of Rindfleisch and Neumann. He taught that both forms of origin occurred. It was quite easy to demonstrate in blood films, which contained plenty normoblasts, *e.g.* from cases of "blood crises" or leukæmia, complete series illustrating how the nuclei of erythroblasts leave the cells and then appear as so-called "free nuclei." It must be specially pointed out that these appearances may be found in specimens in the preparation of which absolutely no pressure has been exercised. On the other hand, no matter how many normoblasts a specimen of blood may contain, it has never been possible to demonstrate Neumann's metamorphosis of the nucleus. The reverse holds good for megaloblasts. Among these cells, but few examples are met with which do not show distinct traces of dissociation of the nucleus and solution of the same. In a good specimen of the blood in Biermer's anæmia, which does not contain too few megaloblasts, an unbroken series of megaloblasts with intact nucleus, through all the stages of karyorrhexis and karyolysis, right down to megalocytes, in full accord with Neumann's description, may be followed.

M. B. Schmidt, Arnold, Helly, Türk, Grawitz, Engel and Bloch, like Ehrlich, have accepted the intermediate position, by acknowledging the occurrence of both forms of denucleisation. These authors, however, differ from Ehrlich with regard to the differential separation of both kinds of cells by the way in which the nucleus is got rid of. Ehrlich ascribed the casting out of the nuclei to the normoblasts and the solution to the megaloblasts.

Clinical differences.—Normoblasts are found almost regularly in every form of severe anæmia which is caused by trauma, inanition, or an extraneous form of organic disease. As a rule they are sparse: after a prolonged search only one such cell may be found. At times, however, one or more normoblasts are found in each field. This is most frequent in acute cases, but may also occur in chronic anæmias, and even in cachectic conditions.

Von Noorden was the first to describe a case in which a large number of normoblasts occurred temporarily in the circulating blood in a case of hæmorrhagic anæmia; the microscopical appearance of the blood, in which there was, at the same time, a marked hyperleucocytosis, simulated that of myeloid leukæmia. As the number of blood cells was found in this condition to be close on double that of normal blood, von Noorden called this blood condition "blood crisis."

The following procedure is recommended for the exact recognition of the blood crises:—

1. Determination of the number of red cells.
2. Determination of the proportion of white corpuscles to red corpuscles.
3. Determination of the proportion of nucleated red to white corpuscles in the dry film. The author recommends the use of the quadratic ocular for this purpose (see p. 32).

For example, if in a case of anæmia, $3\frac{1}{2}$ millions red cells are counted, and it is found that the proportion of white to red is 1 : 100, and of nucleated red to white 1 : 10, there will be 3500 nucleated red corpuscles in each cubic mm. *i.e.*, there will be 1 nucleated to 1000 ordinary red blood corpuscles.

On the other hand, megaloblasts are never found in the blood in traumatic anæmia. They will likewise be looked for in vain in nearly every case of chronic anæmia of a severe type, such as that produced by syphilis, cancer of the stomach, and the like, but they will be found at times in leukæmic blood. The behaviour of the bone marrow in various diseases has been studied from this point of view by a number of observers. Schur and Loewy found megaloblasts in small numbers in two cases of carcinoma, and in one of phosphorus poisoning, and Wolownik found the same in a few cases of carcinoma and one of chronic nephritis. On the other hand, some apparently much milder conditions, in which the diagnosis of an essential progressive anæmia is rendered probable by the anamnesis, aetiology, and general objective condition, are almost always characterised by the appearance of

megaloblasts in the blood. But even in the advanced stages of the disease these cells are not plentiful, and a tedious examination of one or more specimens is frequently required to find them. From this fact a rule has been formulated that the examination of the blood in a case of severe anæmia must not be regarded as complete until at least three or four specimens have been carefully studied under the oil-immersion for megaloblasts.

This clinical difference between the two forms of hæmatoblasts only admits of one deduction, which does not touch upon the much disputed question whether the megaloblasts or normoblasts may be transformed into one another. Normoblasts are found in all those cases of anæmia in which the new formation of blood takes place under normal conditions only in an increased degree, and in a more energetic manner. Nearly all the anæmias, of which the cause is known: acute hæmorrhage, chronic hæmorrhage, anæmia due to inanition, cachexia, blood poisons, hæmoglobinæmia, etc.,—in short, all those conditions which are classed together under the name of secondary symptomatic anæmias, may show this increase of the normal blood formation. In opposition to this, megaloblasts, which represent the embryonal type, are found in those conditions which Biermer distinguished under the name of essential pernicious anæmias, on account of their clinical characteristics. Laache has demonstrated how important this form of blood formation is in pernicious anæmia, by pointing out that in all such cases megalocytes are present, and in many cases in such numbers that they form the majority of the corpuscles. While the red blood corpuscles in the simple forms of anæmia tend to produce smaller forms, the opposite holds good solely and only in the pernicious form. This constant difference cannot be explained as an accidental find, but must be recognised as a regular occurrence. In pernicious anæmia extremely large blood corpuscles are formed. This logical sequence has been confirmed to the full by Ehrlich's demonstration of megaloblasts. All attempts to smooth over the differences between megaloblasts and normoblasts, or to deny that such differences exist, must fail before the

clinical fact that the blood of pernicious anæmia is a megalocytic blood.

The appearance of megaloblasts and megalocytes is a proof that the regeneration of the blood in the bone marrow does not take place normally, but that it follows a type more closely related to the embryonal type. Extreme cases, like the one reported by Rindfleisch, in which the whole bone marrow was found to be full of megaloblasts, are necessarily rare; but the pernicious character may be regarded as sufficiently proved, "if a considerable portion of the bone marrow, and not necessarily the whole of it, reveals megaloblastic degeneration."[1]

It may be said that the megaloblastic transformation is a highly purposeless process, for the following reasons: (1) Because the formation of red blood corpuscles by the megaloblastic process is obviously a much slower one. The fact that megaloblasts only occur in small numbers in the blood, while the normoblasts, as has been stated, frequently are found in very large numbers, speaks strongly in favour of this contention. Accordingly megaloblastic blood crises in connection with anæmia are not met with. (2) Because the megalocytes, which are derived from the megaloblasts, possess in proportion to their volume a relatively small respiratory surface, and on this account must be regarded as a purposeless type in anæmic conditions. This becomes all the clearer when it is remembered that the formation of poikilocytes represents a purposeful process.

The megaloblastic degeneration of bone marrow must be ascribed to the fact that the marrow is subjected to chemical influences, which alter the regeneration type in a purposeless manner. The originating agencies of these toxic processes are to a great extent still unknown. For this reason it is impossible to stop the process, and the disease necessarily terminates in death. This view has received considerable support in the

[1] It may be advisable to emphasise that what has been said with regard to the diagnostic importance of megaloblasts only applies to the blood of adults, the conditions met with in the blood of children, which differs in many respects from that of adults, will be dealt with separately under another heading (infantile pseudo-leukæmic anæmia).

pathology of bothriocephalus anæmia, which, as is well known, offers a favourable prognosis in general. This form occupies an exceptional position among the anæmias of a megaloblastic type, for the sole reason that its cause is known and can be removed. Different individuals react in different ways toward the bothriocephalus, just as they do toward many infective microbes. Some persons are not affected at all by this worm; others show all the appearance of a simple anæmia, possibly with normoblasts; while a third group reveals the typical characters of a pernicious anæmia. The last-named type may be so like Biermer's anæmia that when the cause is not recognised for years there are no means of distinguishing it from the more serious condition. It is therefore not going too far to regard severe bothriocephalus anæmia as a pernicious anæmia with a known and removable cause. Schauman's detailed monograph offers the fullest proof for this conception.

Even if a detailed discussion of this highly important question really belongs to the clinical part of this work (see Vol. II.), one other fact at least should be mentioned in this place, which illuminates the condition very strikingly. Bothriocephalus anæmia and ankylostomum anæmia are two conditions which are very closely allied, clinically speaking. But a remarkable difference between them is brought to light when the blood of persons suffering from these two conditions is examined. Even in its milder forms the blood of bothriocephalus anæmia patients frequently contains megaloblasts and megalocytes, the important characteristics of progressive pernicious anæmia; the blood of ankylostomum anæmia patients, even in fatal cases, merely shows the appearances of a simple severe anæmia (Sahli, Rosenqvist, Schauman, Liernberger). The difference depends on the fact that bothriocephalus stimulates the formation of megaloblasts by means of a specific poison, while ankylostomum acts chiefly by abstracting considerable quantities of blood continuously. In the latter instance, as is the case in traumatic anæmia or the severest forms of post-hæmorrhagic anæmias produced by hæmoptysis or bleeding from a uterine myoma, no megaloblastic

degeneration of the marrow develops. It thus appears that it is not the severity, but the kind of the anæmia, which is characterised by the various kinds of erythroblasts.

The occurrence of megaloblasts in pernicious anæmia must be interpreted in this manner. The megaloblastic degeneration of the bone marrow depends solely on the presence of definite noxious agents, the nature of which is unfortunately not yet known. If it were possible to remove these agencies it would *a priori* be quite certain that the bone marrow could regain its normal normoblastic regeneration type, provided that the disease had not advanced too far. Clinical observation shows this quite clearly in certain cases. It is by no means a rare occurrence for an apparent cure of a megaloblastic anæmia to occur, but after a shorter or longer period the symptoms reappear, and then lead with certainty to a fatal termination. Such cases, and every observer has an opportunity of seeing them, prove that the megaloblastic degeneration as such may regress. In some cases the usual arsenic treatment suffices to produce this result. A definite cure cannot be achieved under these conditions, because the aetiological agent is unknown, and therefore cannot be removed. It may be stated that the prognosis of the megaloblastic anæmias, with the exception of bothriocephalus anæmia, is a hopeless one.

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CHAPTER III

THE WHITE BLOOD CORPUSCLES

The biological significance of the white blood corpuscles is so varied that these cells represent the most interesting chapter in hæmatology. They are motile elements, which reveal considerable changes in response to comparatively slight stimuli.

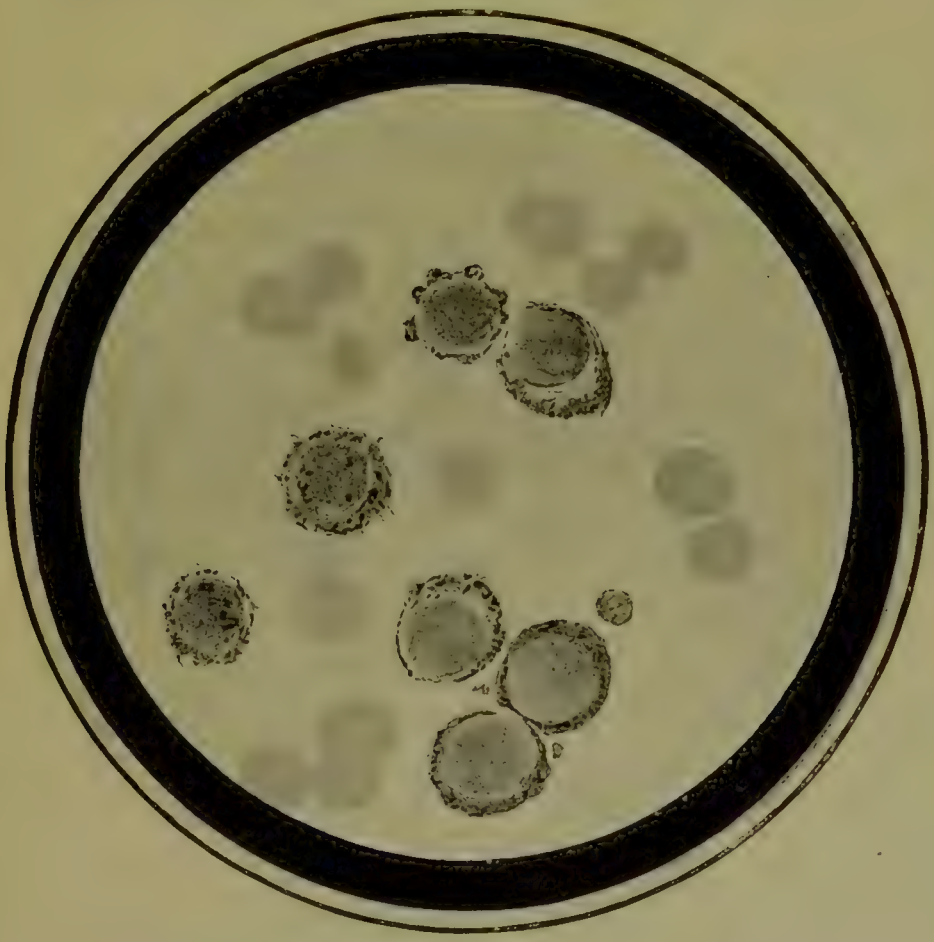

Fig. 2.—Fraying of the Protoplasmal Investment of Lymphocytes; separation of the free Plasma Elements (Plasmolysis). (After a Photograph of a Film from a Case of Chronic Lymphatic Leukæmia.)

Fig. 3.—Nucleoli in the larger Lymphocytes. (After a Photograph of a Film from a Case of Chronic Lymphatic Leukæmia.)

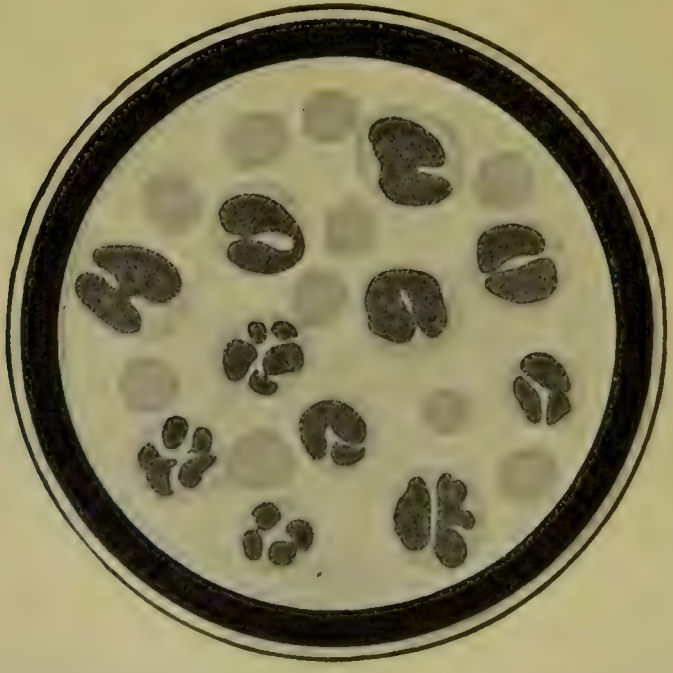

Fig. 4 (From Riedel's *Atlas*).—Transformation of the Nuclei of the Lymphocytes. (Appearance of the Blood in Acute Leukæmia.)

Hæmatologists have only gradually learned to recognise that the white blood corpuscles play an important part in the physiology and pathology of the human organism. It was obviously thought to be unnecessary to ascribe important functions to elements which were present in relatively small numbers in the blood.

Virchow's discovery of leukæmia was the first step toward the recognition of the importance of the white blood corpuscles in pathology. In the next place, the discovery by Cohnheim of the fact that inflammation and suppuration were due to a migration of white blood corpuscles awakened a great interest in the leucocytes. The result of the observations of inflammatory processes was of such a character as to throw a certain light on normal conditions. Thus the fact that in diffuse inflammations large quantities of pus were often formed without impoverishing the blood, as far as leucocytes were concerned, but rather tending to increase these cells, gave rise to the assumption that the site of origin of the leucocytes must be extraordinarily productive, and that in contrast to the red blood corpuscles the small number of white cells was fully compensated by a great capability of regeneration.

Nevertheless it took a long time before the powerful impulse given by Cohnheim yielded tangible results for clinical histology. As has already been mentioned, the methods of blood examination in use up to that time rendered it extremely difficult to form an exact differentiation of the various forms of leucocytes. This fact was responsible for the stagnation in the knowledge of the leucocytes at that time. Even if such eminent investigators as Wharton Jones and Max Schultze were able to describe various types of white blood corpuscles, their work failed to make an impression on clinical practice, because the differential criteria, by means of which they distinguished the types, were much too subtile and were not suitable for clinical examination. Virchow, who discovered leucocytosis, ascribed this condition to an increase in the number of lymphocytes, although, as is now recognised, the polynuclear cells alone

contribute to its production. It was therefore only after the introduction of dry films and staining methods, which rendered the differentiation quite easy, that a great interest was taken in the white blood corpuscles. The astoundingly voluminous literature, especially on leucocytosis, is a proof of this.

The endeavour to divide the various leucocytes from one another, and where possible to trace them back to their separate sites of development, owed its origin to Virchow's recognition of the lymphocytes, and was first thoroughly realised when Ehrlich classified these cells with exactitude. The correctness of this principle has by now been generally acceded, and it has borne rich fruits, especially in clinical medicine. There are still, it must be admitted, a few authors who strive to modify the sharply dividing lines in their endeavour to introduce an alleged simplification. These authors try to show that the various forms of leucocytes are only various phases and developmental stages of the same species of cell. Such views, however, are incapable of replying to the objections raised by morphological or biological criticism, and it is certain that within a short time not a single earnest observer will hold these views.

I. NORMAL AND PATHOLOGICAL HISTOLOGY OF THE WHITE BLOOD CORPUSCLES.

The classification of the white blood corpuscles in general use to-day is that which has been constructed on Ehrlich's suggestions, and morphological, physical, and chemical attributes have been taken into account in formulating it. A definite kind of cell is characterised by a number of properties. These criteria are so constant and reliable that every experienced observer is able to recognise the variety of cell at a glance.

1. **The Lymphocytes.**—These are small cells, being usually of about the same size as red blood corpuscles. The nucleus occupies the greater part of the cell, and is round or oval in shape. The nucleus is centrally placed in the small

obviously young forms, while it is excentrically situated in the older and large cells, so that a broad mass of protoplasm is visible at one side.

The nucleus stains intensely with all the basic dyes, and reveals a dense chromatin network. When suitably stained (the best for this purpose is pyronin methyl-green) a distinct nucleolus is rendered visible, with a relatively broad highly coloured membrane; rarely two such nucleoli are seen. The nucleus often presents a notch, and in older types is not round, but rather of a long oval shape. This cell never shows that peculiar polymorpho-nuclear structure which is met with in other leucocytes, but possesses a more or less round nucleus throughout its whole existence. Only under very rare pathological conditions does the lymphocyte have peculiar lobulated nuclear structures, and it is then known as the "Rieder's cell" of lymphatic leukæmia.

The protoplasm is usually very narrowly developed. In the larger specimens it is broad, and then reveals a basophile reticulum, in which the crossings of the meshes are so prominent that Ehrlich at first regarded them as granules. When examined under a high magnifying power it will be seen that there are no isolated nodules.

A pale area is seen between the nucleus and the protoplasm, in which the protoplasmal reticulum is only very faintly outlined. In this area in every lymphocyte the fuchsinophile granules, which were discovered by Schridde, are placed. They are demonstrable by means of Altmann-Schridde's staining. Since this method of staining has not yet been included in Helly's treatise, it should be described in this place.

(*a*) Method of preparing the Film—

1. The blood is smeared on to the cover-slip in a thin layer.
2. The cover-glasses are then placed for one to two hours in formol-Müller (1 : 9).
3. They are then rinsed first for several minutes with tap water, and then with distilled water

4. They are then immersed for half an hour in the dark in 1 per cent. osmic acid.
5. Next, they are rinsed for a short time.
6. They are then stained with Altmann's anilin acid fuchsin solution (100 c.c. of cold saturated filtered solution of anilin in distilled water with 20 grms. of acid fuchsin.—Filtration). This solution is poured on to the cover-glass, which is then warmed five or six times over the flame until the solution begins to steam, and then put aside until cool.
7. After the dried stain on the edges of the film have been removed with filter paper the films are differentiated with alcoholic picric acid (saturated solution of picric acid in alcohol, 1 part in 7 parts of 20 per cent. alcohol). This solution is dropped on to the film several times, until it appears yellowish or pale yellow.
8. The specimen is then rinsed rapidly with absolute alcohol.
9. It is then passed through toluol or xylol, and
10. Mounted in Canada balsam.

The eosinophile granules are dark red, the neutrophile (amphophile), granules pale brownish red, the basophile granules colourless, like vacuoles. The lymphocytes show perinuclear granules or rodlets of a yellow crimson-red colour.

(*b*) Method of demonstrating the Cell Granulation in Sections (including lymphocyte granules).—The tissues, while warm and fresh, are fixed in formalin-Müller (1 : 9) for twenty-four hours at 36° C. They are then washed for twenty-four hours. Next, they are passed through alcohol, 60, 70, 85, 96 per cent., and absolute alcohol, toluol (in each one hour), paraffin (altogether one and a half to two hours). The sections should be 1 or 2 μ in thickness.

1. The sections are floated on .1 per cent. osmic acid solution for one hour in the dark.
2. Next they are rinsed in distilled water.
3. Next they are stained in Altmann's solution (see above).
4. They are then differentiated with alcoholic picric acid solution, as described above.

5. They are then taken through alcohol, 96 per cent. absolute alcohol, toluol, and Canada balsam.

The sections should appear yellowish, with a trace of red to the naked eye. Microscopically, the cell nuclei are pale brown, the protoplasm yellowish, the granules red (the kind of red varying in different cells).

(*c*) Schridde's Azure II -eosin-acetone Method for Staining Sections.—The fixation may be any of the ordinary methods, *e.g.* formol-Müller (formalin 1 part, Müller 9 parts). Staining with Giemsa (2 drops to each 1 c.c. of distilled water) for twenty minutes. Careful washing, drying with blotting paper, and then treatment for one minute in pure acid-free acetone (Kahlbaum).

The sections are then passed through acid-free xylol or toluol.

They are then mounted in Canada balsam, and kept in the dark.

The neutrophile granules are violet-red, the eosinophile granules red, the mast cells dark blue, the erythrocytes grass green. The myeloblasts show a greyish-blue protoplasm without any granules.

Besides the fuchsinophile granules, some of the lymphocytes possess azurophile granules, which can be rendered visible by means of Giemsa-Romanowski's staining. These granules are met with exclusively in the larger cells. They are at times sparse and coarse and at times numerous, fine granules of a bright red colour. Inequalities and fraying of the contour of the cells are always the artificial results of pressure, and many of the apparently large lymphocytes in the smears are merely the results of squeezing.

The protoplasm possesses no affinity to the neutral and acid dyes save in the case of the larger cells, when the affinity is very slight. It is markedly basophile, and with Giemsa staining it takes on a pure pale blue colour. If the cells have been crushed the protoplasm may remain unstained. The azure granules then appear against an almost white background.

Large lymphocytes occur in the blood of children, but very rarely in the blood of adults. Very large elements are always pathological, and are met with in leukæmia. (Troje erroneously called them "marrow cells" in this connection).

In the blood of adults from 20 to 25 per cent. of the white blood corpuscles are lymphocytes; in the blood of children the number is much greater, and may be as high as 70 per cent.

An increase in the number of lymphocytes is not frequently met with. When it occurs it is spoken of as lymphocytosis or lymphæmia.

2. **Large Mononuclear Leucocytes.**—These cells are relatively very large, being usually twice or three times the size of the erythrocytes. They possess a fairly large oval nucleus, which stains much less intensely with a basic dye than the nucleus of the lymphocyte does. With a suitable dye (hæmatoxylin, Giemsa) they reveal a very delicate slender network of chromatin. The nucleus generally shows a marked tendency to assume a polymorpho-nuclear structure.

The protoplasm is very broad, possesses a close, delicate basophile reticulum, which is extended equally right up to the nucleus, and when stained by Giemsa takes on a dusky greyish-blue (slate-grey) colour. In the meshes an extremely fine neutrophile granulation is seen when the cells are properly stained by the triacid or Giemsa method. This granulation is distributed over several areas of the cells, but not uniformly over the whole cell (young, beginning granulation). In some situations the fine granules are so closely packed that in a well-prepared film some of the edges have a diffuse pink appearance when stained by Giemsa (see Plate II.).

These cells are quite different from the lymphocytes, even if the neutrophile granulation is not taken into account. They belong to the "transition" forms, which will be described later and can only be distinguished from the latter by the nucleus, which scarcely presents any polymorphous character. When they are really well stained with Giemsa the relationship is so clear that it is practically impossible to divide the two classes from one another sharply.

Large and crushed lymphocytes might be mistaken for these cells, if the staining is too pale or the neutrophile granulations are not sufficiently coloured to be distinguishable as such. But

scarcely 1 per cent. of cells is met with which might be confused with the large leucocytes in normal blood, and these are without doubt not to be regarded as lymphocytes from a genetic point of view. That this is so is shown by the fact that intermediate forms do not exist, even under pathological conditions. These cells no doubt belong to the myeloid cell group, and are in all probability formed in the bone marrow out of myeloblasts.

3. **The Transition Forms.**—These cells possess characteristics similar to the preceding form. They are distinguished from them by large, often irregular hollowing out of the nucleus, which may lend the shape of a wallet to it. Another point of difference lies in a somewhat greater affinity of the nucleus to the nuclear dyes, and in the occurrence of more plentiful fine neutrophile granulations in the protoplasm (Giemsa or triacid staining). The second and third groups together represent about 6 to 8 per cent. of all the white blood corpuscles.

It is only possible to distinguish these cells accurately when the film is perfectly stained by Giemsa's method. When this has been achieved it will be seen that azure granules are never found in the large mononuclear and transition forms, and that it is possible to distinguish between the very fine neutrophile granulation and the azure granule in spite of a certain similarity in the tone of the colours. Real difficulty in this respect only occurs when the films are insufficiently stained.

4. **The Polymorpho-nuclear Neutrophile Leucocytes.**—These cells are somewhat smaller than the two forms just described, and may be distinguished by the following characters. In the first place, they possess a peculiar polymorphous nuclear form. The nuclear mass is relatively long and irregularly hollowed out and constricted, in the form of a S, Y, E, Z, etc. A complete breaking up of the nucleus into three or four small roundish nuclei can take place during life as a pathological process. Ehrlich first saw this in a case of hæmorrhagic small-pox; and it is seen frequently in fresh exudations. Formerly the breaking up of the nucleus into several pieces was observed after treatment with the usual reagents, *e.g.* acetic acid, and for

this reason Ehrlich chose the term "polynuclear" for these cells, although it must be admitted that this does not actually fit the condition.

The nucleus stains well with all the nuclear dyes; the protoplasm possesses a marked attraction for the majority of the acid dyes, and is obviously characterised by the presence of a close neutrophile granulation. The protoplasm has an alkaline reaction, which, however, is less marked than that of the lymphocytes. The granulation may be distinctly seen in unstained cells in the form of very delicate non-refractive nodules.

In many pathological conditions, especially in infective processes and suppuration, iodophile substance can be recognised, more especially in the polymorpho-nuclear neutrophile leucocytes. Ehrlich stained films after drying them in the air with iodised rubber solution and later with iodine vapour. Under normal conditions only a very slight brown colorisation in the neutrophile cells can be detected when this technique is employed, although in pathological specimens a very intense diffuse or blotchy reaction may be obtained.

If fresh blood films without fixation and in a moist state be exposed to iodine vapour, as recommended by Zollikofer, all the neutrophile cells without exception, even under normal conditions, take on an intense brownish staining of the iodophile granules.

Save when Zollikofer's vital staining is employed, these granules obviously dissociate very rapidly under normal conditions, but under pathological conditions they reveal a much stronger cohesion.

The reaction depends on a substance which is related to the amyloids, and not on a glycogen.

Neusser's "perinuclear" granules are not preformed elements, but are precipitates of the stains.

The neutrophile cells contain oxydising ferments, and therefore turn guaiacum tincture blue, a reaction which is never met with in the lymphocytes. They possess in addition peptic and autolytic ferments. This was first discovered in autolysis, and later was clearly demonstrated by Stern, as well as by Müller

and Jochmann, who showed that the polynuclear cells made deep dells in albuminous media. There is no doubt that they play a very important part in the organism, quite apart from the well-known phagocytosis.

The number of the neutrophile cells is about 4500 to 5000 per c.mm., *i.e.* about 65 to 70 per cent. of the leucocytes.

The only normal site of production of these cells is the bone marrow.

5. **The Eosinophile Cells.**—These cells are recognised by a coarse, shotty granulation, which shows considerable avidity for the acid dyes and resembles in other respects the polynuclear neutrophiles.

When the cells are lightly stained it can at times be seen that a peripherally placed ring of eosinophile grains take on the dye more intensely than those situated in the interior of the cell. The nucleus does not usually stain very intensely, and is as a rule less lobulated than the nucleus of the polynuclear neutrophile cells. In other respects, however, the nucleus closely resembles that of the last-named cell. These two classes of cell have one property in common, namely, that owing to a considerable contractility they are enabled to pass through the walls of the vessels, and thus into exudations and pus. The eosinophile cells are usually somewhat larger than the neutrophiles.

The granules in an unstained condition show a yellowish glossy fat-like appearance, and on this account these cells can be readily distinguished from the neutrophiles, which possess a much more finely granulated and not glossy appearance in fresh specimens. About 2 to 4 per cent. of the white blood corpuscles are eosinophile cells, which means that there are about 100 to 200 such cells in each cubic millimetre of normal blood. Their site of origin is the bone marrow. Under normal conditions an extramedullary genesis cannot be accepted.

The view which has been expressed by various authors, that the acidophile granules originate in the taking up of hæmoglobin, may be refused altogether. It is impossible for the clinician

to reconcile his knowledge of the eosinophile cells to this view.

Weidenreich's method of proving that the origin of the eosinophile granules is to be sought in hæmoglobin is highly unsatisfactory and has been disproved by Ascoli. Recently, Erich Meyer has proved conclusively that the taking up of red blood corpuscles by macrophages does not produce any eosinophile granules (the Meeting of German Scientists and Physicians, Cologne, 1908). Weidenreich's views have thus been absolutely refuted. But apart from this, the assumption that acidophile granulations took place from a pre-existing structure which possesses a basophile reaction, would render the genesis of hæmoglobin inconceivable.

6. **The Mast Cells.**—These cells occur in normal blood only in small numbers; they seldom reach ½ per cent. Some healthy persons, however, have considerably larger numbers, without any ascertainable cause.

These cells are rather small. Their nucleus is peculiarly polymorphous, and is either shaped like a clover leaf or is quite irregularly lobulated. It takes up basic dyes with but little avidity.

The protoplasm possesses a basophile reticulum. In this reticulum are found moderately large, very basophile granules, which are extremely soluble in water and which in an unstained condition are not glossy. They have a marked characteristic in taking on a metachromic staining, when the basic dyes are mixed with a trace of azure. For example, with methylene-blue they stain violet. This metachromasia is well marked with thionin and with cresyl-violet R (eatin, of the Mühlheim Dye Works). In this last-named mixture the granules are stained almost pure brown.

The staining can be well achieved according to Jenner's directions, since the methyl alcohol protects the granules from solution. Under other conditions the granules dissolve completely or partially in water, and the most weird shapes are left behind. If they are not completely dissolved, mere traces

of the granules may be retained, or even individual grains. When stained by Giemsa, the undissolved granules appear mauve coloured, and the protoplasm on account of the solution of the majority of the granules also takes on this colour.

The normal formation of the polymorpho-nuclear mast cells which appear in the blood takes place in the bone marrow. On the other hand, there are several kinds of histogenic mast cells with round nuclei in the tissues which have quite another genesis. In all probability these two kinds of mast cells have nothing in common.

So much for the colourless cells which are found in the blood of adults under normal conditions.

Pathological Forms of White Blood Corpuscles.

In pathological cases the forms mentioned above may be present in altered numerical proportions, and some other forms which are not found under normal condition at all may be observed. Among these other forms the following are the most important:—

1. **Mononuclear Cells with Neutrophile Granules** ("myelocytes" of Ehrlich).—These cells are generally very large, and possess a relatively large nucleus which stains badly. The nucleus is most frequently centrally situated and is surrounded fairly equally with protoplasm. Apart from the differences in the nuclei, the most marked difference between these cells and the large mononuclear leucocytes of normal blood consists in the fact that the protoplasm of the former contains numerous normal sized (and therefore ripe) and very easily stained neutrophile granules. Beside the large forms of myelocytes, much smaller forms are met with, the size of which does not differ materially from that of the erythrocytes. In addition, there are a number of grades between these two types.

The protoplasm is distinctly but not excessively basophilic. This characteristic decreases as the cells ripen. It also shows a fine basophile reticulum.

The myelocytes are the most common kind of cell of the normal bone marrow. They do not occur physiologically in any other situation. They are generally regarded as the precursors of the polynuclear neutrophile blood leucocytes.

Myelocytes only leave their physiological abode under morbid conditions.[1] This is especially marked when the medulla is affected by an abnormal hypertrophic process (as in leukæmia) or when it works at high pressure, as in many forms of leucocytosis (*e.g.*, in pneumonia, variola, post-hæmorrhagic anæmia).

Myelocytes may be observed as a common find when malignant tumours, and especially carcinomata, are growing in the bone marrow, and intense signs of irritation, probably of a toxic nature, appear in the neighbourhood of the tumour nodules. Neutrophile myelocytes are found in the blood, even when there is no leucocytosis in severe functional disturbances of the bone marrow, *e.g.* in severe anæmias—Biermer's anæmia—in intoxications, and in infections. In these conditions the medulla has obviously lost the power of preventing unripe cells from passing over into the circulation.

The blood in myeloid leukæmia is characterised by a high proportion of myelocytes. A large number of these cells may also be met with in the blood in carcinoma of the bone marrow, and during convalescence from acute infective diseases, *e.g.* croupous pneumonia.

2. **Eosinophile Myelocytes.**—These cells may be regarded as the analogies of the neutrophile cells, and are often found in considerable numbers in myeloid leukæmia. They are only rarely present in other conditions, but may be found in marked eosinophilia, such as in trichinosis and scarlatina, while single cells may be seen in severe forms of anæmia.

Their significance and the causes which impel them to pass

[1] The view which Weidenreich has recently revived, that myelocytes occur in the blood of normal adults, is quite erroneous (*Arch. f. Mikros. Anat.*, 1908, vol. lxxii.), and is actually disproved by Weidenreich's own diagrams. In these diagrams the cells supposed to be myelocytes are without doubt the ordinary large mononuclear leucocytes of Ehrlich. Weidenreich therefore does not even know the normal cells of human blood!

into the circulation are the same as those applying to the other forms of myelocytes. Some of the granules are very frequently deep blue when stained by Giemsa, and not red or reddish brown. Occasionally all the granules show this basophile preceding stage in leukæmia.

3. **Mast Myelocytes.**—These are also analogies of the ordinary myelocytes. They may be present in the blood of myeloid leukæmia, either as small or as large cells. Some of the granules are frequently blue when stained with Giemsa and not mauve. Occasionally all the granules take on this blue colour, since the mauve granulation develops from a more markedly basophile blue young form of granulation. The young mast cell granules differ from the older granules strikingly by their far greater insolubility in water.

4. **Myeloblasts** (Naegeli).—These cells are the precursors of the myelocytes, and contain absolutely no granules. They are the least differentiated cells of myeloid tissue, and are therefore very numerous in the embryonal tissues. Myeloblasts are only sparsely present in the bone marrow of adults, but in certain diseases they may predominate,—for example, in Biermer's anæmia, in other severe anæmias, and in enteric fever. But they are especially numerous in leukæmia, and in the stages just preceding death in chronic myelæmia they may predominate among all the cells of the blood. The same applies also with regard to acute myeloid leukæmia, so that a true myeloblastic leukæmia may be said to exist. All undoubted cases of acute myelæmia reveal this characteristic appearance of the blood, which may be regarded as the expression of such an enormous pathological proliferation that only unripe, little differentiated cells are formed.

The myeloblasts may occur in large or small forms. The former can only be distinguished from myelocytes by the absence of granules.

The nucleus of the myeloblast is relatively large, is roundish or oval in shape, and shows a delicate structure. It stains fairly intensely with the nuclear stains,—at all events much better than

the nuclei of the large pathological lymphocytes. With pyronin-methyl-green staining and also with properly managed Giemsa, it can be demonstrated that there are almost always several nucleoli present; as a rule there are three or four, while at times there are more. With Giemsa the nucleoli stain blue, and are therefore easy to recognise and to distinguish from collections of chromatin. In chronic myeloid leukæmia these nucleoli may be seen very distinctly as blue rings in the nuclei when stained by Giemsa, while in the ripe myelocytes nothing of this nature can be detected.

The protoplasm is reticular and definitely basophile. The network is continued right up to the nucleus without any interposition of a free areola. There are no azurophile or fuchsinophile granules of Schridde. As a rule, however, cells are met with which are otherwise similar in every respect to the regular myeloblasts without granulation but which show beginning neutrophile granulation. These are the intermediary forms between the myeloblasts and myelocytes. The well-marked basophile character of the protoplasmic reticulum decreases and the nucleoli disappear as the fine neutrophile granulation increases. These intermediate forms may be distinguished from the large mononuclears and transition forms by their round or oval nucleus with nucleoli, and by the fact that the reticulum of the protoplasm stains blue rather than greyish blue.

Myeloblasts are always met with in myeloid formation outside the bone marrow, and especially in embryonal livers. They can be readily demonstrated in sections by means of Schridde's or Fischer's staining methods, and their characteristics can be recognised as contrasted with the lymphocytes.

The fact that these cells cannot possibly be lymphocytes is proved by the complete absence of fuchsinophile granules, and especially by their relationship to myeloid formation. Apart from this, it must be recognised that, histologically and histogenetically, they are the direct antithesis of the cells of lymphatic tissue.

It will thus be seen that these cells must be separated

completely from the lymphocytes on biological grounds. Myeloblasts only occur in the blood and tissues in severe disturbances of the myeloid system, and are then accompanied by myelocytes and their descendants; they are further often associated with nucleated red blood corpuscles. In severe lesions, or when there is rapid proliferation of the medullary tissue, they pass over into the circulation in steadily increasing numbers. Under these conditions the forms intermediate between these cells and the myelocytes are met with regularly in large numbers.

Morawitz and Rehn have produced experimental evidence to prove that the myeloblasts are the most indifferent cells in the medullary tissue. They were able to produce artificially an aplastic anæmia by repeated large bleedings, until the red medulla became absolutely incapable of producing any more erythrocytes and the leucocyte formation was limited almost entirely to non-granulated myeloblasts.

The myeloblasts are distinguished from the atypical pathological large lymphocytes by the absence of a fuchsinophile perinuclear zone of granules, by the more intense triacid staining of the nucleus, and by the network of basophile protoplasm, which reaches right up to the nucleus.

Even if it must be admitted that it may be difficult in some instances to recognise the myeloblasts with certainty, or even impossible when the differential staining is not sufficiently distinct, there can be no possible doubt that non-granulated cells of the myeloid system actually exist, which have nothing whatsoever to do with lymphocytes. This has been definitely proved by the histology of the hæmopoietic organs, by the study of the developmental conditions, and by the biological behaviour of the cells themselves.

The myeloblasts, considered from a theoretical point of view, are of very considerable importance. If they were identical with lymphocytes the chief support on which Ehrlich built up his classification of the leucocytes would fall to the ground. It would be impossible to support in principle a division of the

two leucocyte-forming organs and the cells which these organs produce, *i.e.* the dualistic view.

It is not surprising that the opponents of Ehrlich's doctrine refuse to recognise the myeloblasts. If this were sound this chapter of hæmatology would return to the chaos in which it was formerly placed, and the leucocytes would represent various phases of one kind of cell.

Embryology, morphology, histology, and biology, however, demand a sharp dualistic division in no uncertain voice, and assert on principle the difference between the myeloblasts and the lymphocytes.

The large mononuclear cells and the transition forms of the blood are not myeloblasts, and can be distinguished from these cells by their totally different nuclei, in which no nucleoli are demonstrable by means of Giemsa's staining, and by their neutrophile granulation; but it must be recognised that these normal cells of the blood are derivatives of the myeloblasts.

5. **Stimulation Forms** (Türk) = **Pathological Myeloblasts** (Schridde, Naegeli).

Under pathological conditions, especially in inflammatory leucocytosis, and also in anæmia, tumours, etc., the blood may contain cells which are characterised by a highly basophilic protoplasm (deep blue when stained by Giemsa; bright red with pyronin-methyl green; dark reddish brown with triacid).

These cells are usually large, and may be very large, although small examples do occur.

The nuclei are round or oval, take on the stain intensely, and in structure look exactly like the nuclei of myelocytes and not of lymphocytes. The author has never met with nucleoli. A radiating structure of the nuclei is not present. The nuclei usually lie excentrically placed. The protoplasm is markedly basophilic, and often shows well-marked vacuolisation.

These cells have no connection with the true lymphocytic plasma cells, in spite of the views which were formerly held. That this is so is proved by the absence of a radial nuclear structure, a perinuclear zone, and fuchsinophile granules. At

first sight the two cells appear to be very similar, on account of the well-marked basophilic character and the presence of vacuoles in the protoplasm. It was formerly held that they were related to the nucleated red cells, but this is certainly incorrect. On the contrary, it must be recognised that these cells are pathological myeloblasts. Their connection with the myeloid system, which can be shown morphologically, suggests this, and the view receives further support by a study of the biological behaviour of the cells. They occur as a rule together with myelocytes, especially in leucocytosis, as in croupous pneumonia. They are not infrequently met with in small numbers in other conditions.

6. **Pathological Lymphocytes.**—In lymphatic leukæmia and especially in the acute forms, peculiar cells pass into the blood, which of necessity must be regarded as pathological since they have no physiological analogies.

They resemble the large lymphocytes, often showing a wide protoplasmic band, with all the characteristics of lymphocytes. On the other hand, azure granules are but rarely present, and may be entirely absent. The protoplasmic reticulum and the perinuclear zone, however, are particularly prominent.

The nucleus is poor in chromatin, and therefore takes up the stain badly, so that ordinary triacid staining is insufficient. The staining is best carried out with Giemsa. The author has come across one or two nucleoli in the nuclei in all his specimens. Azurophilic granules are frequently met with.

Beside possessing the characteristic of occurring as giant cells, there is a tendency of the nucleus to form a peculiar coarse hollowing out and lobulation, so that actual polymorphous nuclei result. The nuclei, however, have no similarity to the slender, drawn-out nuclei of the leucocytes. These forms are usually termed "Rieder's forms" (see Fig. 4, p. 88).

The origin of these cells is placed in the lymphatic system, as has been shown by histological study of the organs. The fact that those forms, lying intermediate between the myeloblasts and the myelocytes which are so frequently met with in

myeloblastic leukæmia, are never found in these organs speaks against a possible connection of these cells to the myeloblasts. The pathological lymphocytes may be observed in ordinary lymphatic leukæmia of many years' standing, albeit in small numbers. There are further types of leukæmia in which the small lymphocytes are at first increased in number, and later these cells become larger until the pathological forms are seen. Under the effect of sepsis and other factors, the predominating large forms and Rieder's cells may disappear again, and practically only small cells remain (see Fabian, Naegeli, Schatiloff). The changes connected with these cells are extraordinarily great.

The form dealt with in this section is present almost exclusively in lymphatic leukæmia and lymphocytomata.

7. **Plasma Cells.**—These cells are extremely rare constituents of the circulating blood. They may occur in plasma-cell leukæmia (Gluzinski and Reichenstein and the author), in plasma-cell myelomata (Aschoff-Schridde), and as curiosities or single types, *e.g.* in mastitis and lymphatic leukæmia (Naegeli). They are characterised by a radiating nucleus which is rich in chromatin, and as a rule excentrically placed, and which contains one or two nucleoli, by a prominent perinuclear zone, by the existence of protoplasm, which is extremely basophilic and which shows large vacuoles, and lastly by the presence of Schridde-Altmann's fuchsinophile granules.

Plasma cells are very commonly met with in the tissues. In this situation they exist either as small lymphocytic cells with dark radiating nuclei, or as large lymphoblastic cells with pale nuclei, which do not show distinct radial structure.

The origin of the plasma cells is no longer uncertain. They are pathological descendants of the lymphocytes. This is proved by the presence of Schridde's fuchsinophile granules. In the earlier definitions Unna did not limit the use of the term plasma cell nearly as sharply as it is now limited; he included all those cells which gave the so-called plasma reaction, *i.e.* which showed an intensely basophilic protoplasm. This criterium does not suffice the present needs, since it would

include cells of a very varied origin and even young connective-tissue cells.

Plasma cells, as they are regarded at present, are well characterised cells which are exclusively derived from lymphatic elements.

It is unnecessary to discuss in this place the importance which these cells possess for the various tissues of the body.

It must not be supposed that the foregoing is a complete list of the abnormal forms of the white blood corpuscles. There is no need to deal specially with variations of the size of the cells, which affect the polynuclears and the eosinophiles chiefly, and which lead to the formation of dwarf and giant forms. Even when the difference in size is very considerable these cells always possess sufficiently marked characteristics to enable the observer to recognise them as cells of the given type.

In addition to the forms mentioned, there are cells which contain granules possessing absolute or partial basophile characters. This occurs more especially in the eosinophile myelocytes with mixed acidophile and unripe basophile granulation, but which in contrast to the mast cells do not show any metachromic granulation. Such cells are frequently met with in leukæmia.

At times the individual granules of the neutrophile leucocytes reveal weak basophilic characters, in the form of basophile young forms.

Pathological changes can be recognised in the development of neutrophile leucocytes, especially in severe infective processes, when the granulation of the protoplasm is either absent or very badly developed, while the nucleus is well formed and shows the type of a slender polymorphous formation.

Abnormal appearances of the nuclei are met with still more frequently in infective diseases. In these cases the lobulation of the nucleus is less well marked than in the cells of normal blood. Arneth has studied these variations very minutely and believes that they are young elements, because the polymorphonuclear cells are derived from the round nucleated myelocytes. He divided the neutrophiles into classes according to the

number of segments of the nucleus, and termed the condition, when the segments were less numerous than normal, as a "sinistral asymmetry."[1] Arneth's conclusions are very far reaching, not only with regard to diagnosis but also with regard to prognosis, treatment, and some aspects of the doctrine of immunity. Up to the present, both confirmatory and adverse criticisms have been expressed. Arneth attempted to disprove the latter, some of which, it must be admitted, are very weak.

In the first place, it must be admitted that the young elements show indistinct lobulation of the nucleus, and that it is extremely difficult to classify them: two competent observers may differ as to the number of segments. The more marked the nuclear staining is, the greater will be the difficulty in dividing them into individual classes. The author has often been unable to arrive at any definite conclusion in a large percentage of cells, especially when he has employed Giemsa staining.

Quite apart from the fact that some observers have included large mononuclears and transition forms in the first and second classes, actual difficulties in the determinations arise for the following reasons.

In the first place, for example, bisegmented nuclear forms may be closely connected with myelocytes, as Arneth supposes, but in the case of more segments being present in the nuclei it is difficult to prove such a regular stage series in the development as Arneth's classification assumes. In the next place, there are undoubtedly other possibilities which might produce an apparent simplification of the lobulation of the nuclei. It is by no means uncommon for pathological leucocytes to appear in the blood, possessing but slightly lobulated nuclei, which do not belong to the early stages of the developmental series. The segment in these cases are small and stain darkly, are blotchy or swollen, and do not possess the delicate lightly stained chromatin network of young elements.

[1] The term "Verschiebung nach links" has not yet, as far as the translator is aware, been translated into English. After consultation with hæmatologists and mathematicians, the term "sinistral asymmetry" has been decided upon.

Even if there are many possibilities under which imperfectly lobulated forms of nuclei occur, it must be admitted that the occurrence of such cells is abnormal and therefore worthy of notice. The author, however, does not feel justified in going nearly as far as Arneth does in his deductions, especially because the methods of examination are not very reliable.

II.—ON THE SITE OF ORIGIN OF THE WHITE BLOOD CORPUSCLES.

It is of the utmost importance, for the purpose of gaining a clear insight into blood histology, that exact impressions should be gained with regard to the formation of the blood. It is necessary to inquire whether and in what degree the three systems: the lymphatic glands, the bone marrow, and the spleen, all of which undoubtedly are intimately associated with the blood, participate in its formation.

The most direct method of deciding this question experimentally, *i.e.* by eliminating the organ concerned, is unfortunately only applicable in the case of the spleen. The importance of the lymphatic glands and the bone marrow, which cannot be eliminated *in toto*, must therefore be estimated by histological and clinical investigation. It is, however, only possible to gain a clear insight into this and similar, equally important questions by combining animal experiment, histological examination, and especially clinical biological observation, and carefully registering the results in a large number of cases. It cannot be too strongly urged that it is essential that every one who wishes to carry out hæmatological researches should first gain a general experience by examining a large number of specimens, so that mistakes may be avoided. In many instances an attempt has been made to cover a want of experience by substituting a careful study of the literature; but the histology of the blood has not been advanced thereby in the least. A characteristic of this kind of work is found in the habit of drawing far-reaching conclusions involving the whole pathology of the blood from the examination of a

single case or from one independent observation. An example of this may be quoted in Troje's publication of the details of a case in which he did not recognise the lymphatic character of the leukæmia, and on this account regarded it as an example of myelogenous leukæmia, thereby rendering everything that had been previously established with regard to this subject nugatory and turning it all upside down. Another instance was the definition of the term hyaline medullary cell solely on the basis of the finds in bone marrow smears, from which it was considered that these cells are the precursors of all red and white corpuscles (Grawitz). This form of medullary cell is never found in sections. It is merely an artificial appearance, crushed myeloblasts, the basophilic nature of the protoplasm of which has been lost in the crushing. Further, it is just as difficult to avoid falling into error when conclusions are based entirely on animal experiment without any controlling by clinical experience, as has been done in numerous articles by Uskoff. The clinician rather than the anatomist or the physiologist is capable of offering information on these matters.

It is true that the development of granule staining in sections has gone so far that embryology and minute histology as well as biological clinical studies are capable of revealing valuable information. In the combination of all these branches of investigation, and in the supplementing of one method of research by another, the majority of the questions are being explained at present. The "peripheral" hæmatologist has no longer any justification for his existence.

It is always absolutely necessary to supplement clinical investigation by embryology and histology for advance in histogenetic problems. Many points have been clearly settled in this way, and the science of hæmatology is certainly approaching some definite final conclusions.

It will therefore be realised that the views of some authors have become unworthy of being taken into consideration when their studies have been limited to the peripheral blood and possibly smear preparations from the organs.

(*a*) The Spleen.

The question whether the spleen produces white blood corpuscles has been a burning one since the early days of hæmatology.

Attempts were first made to prove the participation of the spleen in the formation of the white blood corpuscles by counting these cells in the afferent and efferent vessels of the spleen. It was even suggested that the increase of cells in the vein as compared with the artery could be accepted as proof of the blood-forming power of the spleen. The results of these countings, however, are extremely variable, and while some observers found an increase in the veins, others found the reverse. It is now realised that such a coarse method of attacking the problem is of no practical value.

Certain facts have been brought to light by recent researches. It has been found that, after removal of the spleen, some of the lymphatic glands become more fully developed, while the changes of the thyroid gland, which have been observed by some investigators, cannot be regarded as constant.

Attention must further be called to the blood observations which Mosler, Robin, Winogradow, Zesas, Staehelin, and others carried out with animals and human beings from whom the spleen had been removed. These experiments show that after the lapse of a considerable time a definite leucocytosis occurs. Professor Kurloff carried out some exhaustive experiments in Ehrlich's laboratory in 1888, by means of which he was able to study the behaviour of the blood after removal of the spleen.

These experiments have been minutely described in the first edition of this work, and will therefore only be sketched in outline in this place.

The blood of a normal guinea-pig contains:—

1. Polymorpho-nuclear leucocytes with pseudo-eosinophile granulation, functionally analogous to the neutrophiles of the human subject, 40 to 50 per cent. They are derived from the bone marrow.

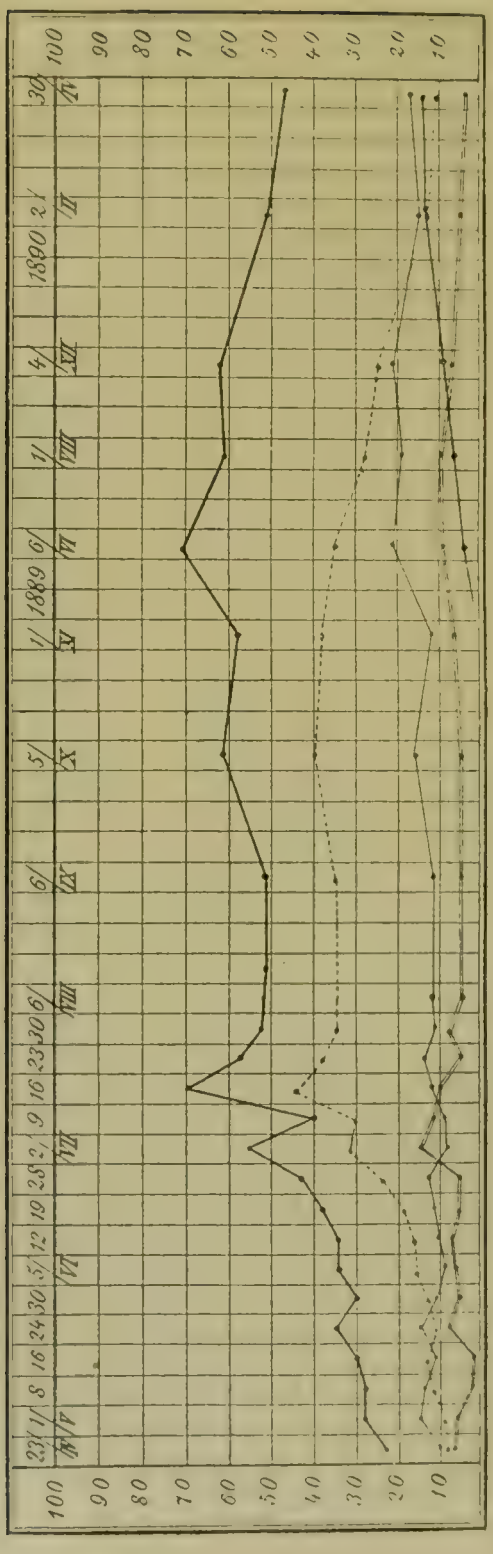

FIG. 5.—CURVE TO EXPERIMENT I.—(In the curve the figures in the scale are adapted for comparison of the various forms of blood cells).

The thick line indicates the number of leucocytes generally.
The blue line indicates the lymphocytes.
The thin line = the number of nucleated, real pseudo-eosinophile cells.
The double line indicates the large mononuclear cells.
The red line indicates the eosinophile cells.

2. Polymorphonuclear eosinophile cells, about 1 per cent.

3. Nigrosinophile cells, analogous to the eosinophile cells, but the granules of which take on the colour of nigrosin from the eosin-nigrosin mixture in preference to the red. They take on a darker colour when stained by triacid.

4. Cells with vacuoles, 15 to 20 per cent.

5. Lymphocytes, 30 to 35 per cent.

Kurloff, in his extremely careful and laborious examinations, determined the total number of leucocytes and then the percentages of the absolute number of pseudo-eosinophile, neutrophile, eosinophile, and vacuol-

ated cells, as well as of the lymphocytes. He was thus able to prove that in uncomplicated cases of removal of the spleen, in which all inflammatory processes which involve an increase of the polynuclear neutrophile corpuscles were excluded, a gradual increase limited to the lymphocytes up to twice or three times the original value is noticed, while the numbers of all the other elements remain absolutely unaltered.

The increase in the number of lymphocytes occurs during the course of the first year after the removal of the spleen. This increase must be regarded as the expression of a hyperplasia of the lymphatic glands, and especially of the mesenteric glands. The loss of the splenic function is thus in part compensated by the lymphatic system.

The pseudo-eosinophile cells show a temporary increase after the operation, but no marked variations have been noticed in the transition forms.

In the second year after the operation a very considerable increase in the number of eosinophiles is constantly observed.

Kurloff's experiments thus prove that the spleen of the guinea-pig only plays a minor part in the formation of white blood corpuscles, and that after splenectomy, compensatory functions are assumed during the first year by the lymphatic glands. In the second year a considerable increase of the eosinophile cells takes place. It is necessary again to point out that the spleen has nothing to do with the formation of the pseudo-eosinophile polynuclear cells, which are the analogies of the polynuclear neutrophile cells of human beings.

It is necessary in the next place to inquire how observations on human beings compare with Kurloff's observations, which after all might be regarded as peculiarities of the species of animal.

Absolutely analogous conditions can be found in those cases of healthy persons who, as the result of trauma, have been subjected to splenectomy. Unfortunately, such cases are extremely rare, but it would be of great value if the changes in the blood could be studied systematically for several years in such cases.

The observations made up to the present have led to the

following results. A lymphocytosis has been observed after the operation, which at times was of considerable duration and at times merely temporary. In the latter case the increase was probably only an after-effect of the operation, and corresponds to the experience that the diminished formation of lymphocytes after operative interference is overcompensated during convalescence.

In a few cases a slight increase of the eosinophiles has been observed. This too could be regarded as a post-infective or post-toxic process, and cannot be regarded as justifying the conclusion, at present at all events, that the bone marrow in human beings takes on a vicarious function. It is not an infrequent find that a considerable increase in the eosinophiles is present in splenic tumours, but the cause of this is probably to be sought in the disease itself and not in the loss of splenic function.

It is, however, essential that further careful observations in human pathology are needed to clear up this question.

In the meantime, direct histological examination has become applicable for the study of the participation of the spleen in the formation of the blood. By means of modern section staining a perfectly clear insight into the conditions is rendered possible by these methods, at all events as far as the majority of the points under discussion are concerned.

These examinations show that the normal human spleen does not contain any nucleated red blood corpuscles. Only a few authors claim to have seen a few such cells. It can therefore be definitely stated that the human spleen during adult life takes no part in the production of erythrocytes, or at all events no material part.

The same applies to the occurrence of myelocytes, the precursors of the polymorpho-nuclear blood cells. These cells are not met with in stained sections, although a few authors state that single examples of these types have been seen on rare occasions.

The spleen has therefore nothing to do with the normal formation of the polymorpho-nuclear leucocytes; this takes place exclusively in bone marrow.

On the other hand, one origin of the lymphocytes is found in the Malpighian bodies, and there can be no doubt that a certain proportion of the lymphocytes of the blood proceed from the spleen. The spleen would, according to this point of view, belong to the lymphatic system.

The cells of the splenic pulp, however, must still be taken into consideration. Up to the present the significance and character of these cells are quite unknown, and information on this point can only be obtained by means of the most delicate methods of cell analysis.

The normal functions of the spleen must be regarded as including a process whereby a portion of the used-up white and red blood cells are completely disintegrated and the utilisable material is used for the reconstruction of new cells. Spodogenous tumours of the spleen are consequently met with in many diseases (from σπόδος = fragments).

Comparative anatomy and histology, however, teach that in some of the lower vertebrates and in many mammalians, *e.g.* mice and rabbits, the spleen fulfils a much more important part in the formation of blood. Nucleated red blood corpuscles may be found in nests, and the same applied with regard to the myelocytes, so that as far as these animal species are concerned there is no doubt that this organ possesses a blood-forming activity.

With regard to human beings, observations of this kind have only been made under embryonal or pathological conditions. These observations have been made quite recently, although some finds of an unconvincing nature have been reported during the past two decennia. It is now known that the human spleen in the early stages of embryonal life at first exercises an erythropoietic and myeloid activity exclusively (Naegeli, Schridde). In a fœtus of from $10\frac{1}{2}$ to $11\frac{3}{4}$ inches length the spleen is composed of almost pure myeloid tissue, so that even an experienced histologist would diagnose bone marrow at first sight from a smear. Later on this function diminishes little by little, until the lymphatic structures are developed in the Malpighian bodies,

and at the time of birth the spleen has lost nearly all traces of its former myeloid-erythropoietic functions.

Under pathological conditions, however, the human spleen may again harbour erythroblasts and myelocytes, as if it had reverted to its embryonal habits. Under these conditions the cells mentioned are not merely washed into the organ. Actual formations and even quite extensive transformations can at times be discerned, in which the lymphatic tissue of the follicles is reduced or even stifled and substituted.

Observations of this nature have been reported in such great numbers during the past few years that it is impossible even to name the individual authors responsible for them. Suffice it, therefore, to mention that this form of transformation in the structure and function of the spleen actually takes place. The conditions under which this occurs include the infective diseases, severe anæmias of various origins, malignant tumours, provided that they have led to anæmia or destruction of the bone-marrow tissue (carcinoma of the medulla of bone), and especially leukæmia and the allied conditions.

Experimental research did not have any great difficulty in discovering some absolutely analogous histological appearances. A complete transformation of the spleen was produced in experimental anæmia caused by blood poisons, by artificial infections, and by exposure to X-rays (K. Ziegler). It is proposed to discuss how such a surprising phenomenon may be brought about later on.

Consequently it is necessary to adhere to the view, which Ehrlich formulated with considerable precision some years ago, that the normal human spleen does not participate in the formation of the red blood corpuscles and of myeloid tissue; but, in opposition to the older views, this formation takes place in the spleen frequently and at times extensively under pathological conditions, like a reflection of embryonal times.

(*b*) The Lymphatic Glands

Since it is impossible experimentally to eliminate all the lymphatic glands from taking part in the formation of blood, it

is necessary to depend entirely on clinical and histological observations for the purpose of obtaining information on this subject.

Since Virchow defined lymphocytes, the identity of the lymphocytes of the blood and the lymphocytes of the lymphatic glands and those of other forms of lymphatic tissue, be they large or small types, has not been questioned. This identity is proved by the complete correspondence of the general morphological characters, and of the tinctorial peculiarities both of the protoplasm and of the nucleus.

With regard to the granules in the lymphocytes, the proof of the identity of the blood lymphocytes and those of the lymphatic glands can be further clinched by the demonstration of Schridde-Altmann's fuchsinophile perinuclear granulation, which is possible in every lymphocyte in lymphatic glands. In the same way, some of the lymph cells show azure granules. The identity is therefore complete in this respect also, and since both these forms of granulation do not occur in any other cells save the lymphocytes, the correspondence may be regarded as absolutely proved. Now, since similar cells (myeloblasts) occur in the parenchyma of the bone marrow, which, however, do not show these specific characters (Schridde, Naegeli), it follows that the bone marrow does not produce any lymphocytes normally. The differential distinction between the two cells is shown in this way. Only a few isolated lymphocytes are met with in the sheaths of the vessels of the medulla.

It is characteristic of the advance in hæmatology due to Ehrlich's teaching that a direct proof of identity and new formation of cells is now forthcoming, while formerly, owing to the absence of good section staining, the observer was forced to rely on indirect methods which were far less certain.

Ehrlich based his doctrine of the origin of the lymphocytes from the lymphatic glands chiefly on biological grounds. He pointed out that when extensive areas of lymphatic tissue were eliminated by new growths and similar changes, the number of lymphocytes was sensibly diminished. This fact has since

been confirmed by a number of authors. For example, Reinbach described several cases of malignant tumours, especially sarcomata, in which the percentage of lymphocytes, which is usually about 25, was very materially diminished: in one case of lympho-sarcoma of the neck these cells only represented 0·6 per cent. of the total number. The author observed a case (published in the J. D. Chotimsky, Zurich, 1906) of general enlargement of the lymphatic glands, in which, during the course of two years, the absolute value of the lymphocytes varied in a large number of counts between 300 and 500, as compared with the normal 2000. In spite of the extraordinary generalisation of the process, which suggested an aleukæmic early stage of a lymphatic leukæmia, this diagnosis could be definitely excluded on account of the biological-functional phenomena, and a process of destruction of the active lymphocyte-producing tissue had to be assumed. The post-mortem examination and subsequent histological investigation showed that the case was one of a tuberculosis, having the course of a pseudo-leukæmia and leading to complete induration and scarring of the glandular tissue.

These appearances can be explained quite readily and naturally on the assumption of the elimination of the lymphatic glands. It is difficult to say how the supporters of the view that the lymphocytes are the precursors of all white blood corpuscles can explain these facts. In accordance with this view, it would have to be assumed that the small number of lymphocytes in such cases would be accounted for by supposing that an unusually rapid transition into the elder forms, the polynuclear elements, had occurred, or, to adopt Uskoff's vernacular, that a premature getting old of the lymphocytes had taken place.

Further proof that the blood lymphocytes are derived from the lymphatic glands can be obtained from those cases in which an increase of lymphocytes in the blood is found. These lymphocytoses are of rare occurrence as compared with other forms of leucocytosis. In the first place, it can be seen that certain conditions, in which a hyperplasia of the lymphatic

gland apparatus occurs, are associated with an increase of lymphocytes in the blood. Ehrlich and Karewski examined a long series of typical cases of lymphoma malignum (they did not publish their results). They noted a regular lymphocytosis which was very considerable in some of the cases and had almost a leukæmic character.

On the bases of these results, Ehrlich and Wassermann (*Dermatologische Zeitschrift*, 1894, vol. i.) formed the diagnosis of malignant lymphoma during life in a case of a rare form of skin affection. The blood showed an absolute increase, which was limited to the lymphocytes. No swelling of the lymphatic glands was ascertained by palpation. The post-mortem examination revealed that the retroperitoneal lymphatic glands were swollen to the size of a fist.

In cases of this kind there is a marked increase of production of lymphocytes in the whole lymphatic apparatus, as can be proved by histological preparations. Some parts of the apparatus no doubt are but slightly affected, but the proliferation is well marked in others. This means that there is a system affection of the lymphatic apparatus, which in view of its nature is termed lymphocytomatosis. This affection may last for several years and may pass on to a true lymphatic leukæmia, from which it differs only in point of extension.

It is therefore possible to gain information with regard to the nature of certain affections of the lymphatic glands on biofunctional considerations, and at the same time to determine essential differences between the various forms, even when the clinical appearances do not serve to clear up the matter. It must, of course, be realised that these considerations can only be regarded as correct and utilisable if the anatomic histological premise corresponds to fact, namely, that the lymphatic glands are the sites of origin of the lymphocytes.

Proof has recently been adduced that in the earliest embryonal stages, before the lymphatic apparatus has been developed, no lymphocytes are found in the blood, and that the blood then contains cells of the myeloid series exclusively.

It is, as would be expected, extremely difficult to say how large a proportion of the lymphocytes is derived from the lymphatic glands. The lymphatic follicles of the intestinal tract and, as has already been mentioned, the spleen undoubtedly supply the blood with true lymphocytes. But the clinical experiences made in cases of destruction of lymphatic glands, to which allusion has been made, goes to show that the preponderance of these cells originate in the glands. If this were not so it would be extremely difficult to explain the very low values which have been observed and which persist for years.

A marked diminution of the lymphocytic value is met with frequently and with considerable regularity in acute diseases, and especially in the early stages of the infective processes. This diminution is resolved in the later stages by an increase in the absolute numbers which may attain a quite considerable degree during convalescence. Under these conditions, even if it cannot be said that histological changes are not present, the changes must depend to a large extent on functional factors, such as the toxic inhibition of the cytogenesis and a consequent hyperactivity, according to general biological laws. It is just the late increase which cannot possibly be explained otherwise than as a biofunctional process.

Consequently the phenomena of hypo- and hyper-lymphocytosis must always be judged with caution, and it is essential in all cases to think of the possibility of functional changes rather than of gross anatomical lesions, although even when the former are active the latter need not be excluded. An example of this may be quoted in the later stages of pertussis and of enteric fever, when enlargement of the lymphatic glands is met with. This enlargement should be regarded as the anatomical substratum of the existing increase of lymphocytes.

Chemical substances induce a preliminary diminution in the number of lymphocytes in the blood as the result of a functional process (toxic inhibition of the cytogenesis), while, as is well known, the myeloid system usually reacts to a stimulation of the

functions of the organs by a marked leucocytosis. An increase of the lymphocytes only takes place later as an after-effect, in accordance with biological laws. This reactive increase continues beyond the normal niveau as the function recovers itself.

Hitherto only one substance has been mentioned in literature which is stated to be capable by itself of producing a lymphocytosis. Waldstein reports that he has succeeded in inducing a lymphæmia by injecting pilocarpine. On increasing the number of injections he obtained a progressive character of the changes.

Observations of this kind do not prove much, since an aleukæmic lymphocytosis may at any time pass over to its leukæmic stage and then assume a progressive character. It appears to be exceedingly doubtful whether pilocarpine can induce a primary and not secondary functional lymphocytosis on an unprepared soil, and would have to be proved by repeated careful investigation before it could be accepted.

The production of a lymphocytosis therefore depends on absolutely different causes to those which act in producing the ordinary leucocytosis in which an increase of the neutrophile elements is found. As Ehrlich pointed out long ago, the chief difference is found in the fact that chemotactic functions play a principal part in the production of leucocytosis, and that this exercises a distant action on the bone marrow. In lymphocytosis this chemotaxis is not present, or is only present to a very slight extent. A primary increase of lymphocytes is therefore unknown.

On the other hand, it can be said at present that secondary lymphocytosis, which is seen in the later stages, cannot be regarded solely as the result of an increase in the lymph circulation, which would mechanically cause a larger number of the elements to be washed out of the lymphatic glands.

Clinical observation has taught that a functionally augmented activity sets in during recovery after the stage of diminished function, which is usually a sign of toxic inhibition, and that the hyperlymphocytosis is then the expression of a true increase of

function. It is therefore necessary to add the functional explanation to the mechanical explanation formulated above. In the same way, in severe pathological affections involving an actual proliferation of the lymphatic tissue, as in lymphocytomatosis and lymphatic leukæmia, a marked increase of activity of the tissue takes place and not a mere mechanical washing out.

The lymphocytes do not play any part, as a rule, in inflammatory processes, and are not met with in the inflammatory foci. This corresponds to the absence of a chemotactic attraction.

Neumann described many years ago a highly interesting experiment bearing on this question. He produced an abscess in a patient who was suffering from lymphatic leukæmia, and whose blood contained a very small number of polynuclear cells. The pus was found on examination to consist exclusively of polynuclear leucocytes; not a single lymphocyte was found in the discharge, although the blood was full of these cells.

The same results have been obtained each time this experiment has been repeated.

If, in spite of this, lymphocytes leave their vessels actively, and this has been observed several times (Schridde, Helly, and others), quite a different cause must prevail to that which prevails in ordinary leucocytosis.

Histological examination of nearly all fresh inflammatory processes in which the polynuclear elements alone are found in the inflammatory tissue also yields results conforming to this view. Under exceptional conditions lymphocytes may pass out of the vessels in the earliest stages of fresh inflammations. There must in this case be special, undoubtedly different attractions to those which act on the leucocytes. In the case of migration of lymphocytes a local action of the vascular wall and the tissue in its immediate environment must take place, and not a distant action, which reaches as far as the blood-forming organs. It is well known that in the later stages of

inflammation small cell infiltration appears, which consists apparently of lymphocytes. But this does not prove that these lymphocytes have migrated from their vessels to the site of inflammation. It is unnecessary in this place to enter into a discussion of the controversy which has been engaging the attention of a number of hæmatologists with regard to this question. It will be sufficient for the present to mention that all the investigators have considered, in the first place, the possibility of a new formation of the cells *in situ*. Evidence of this occurrence has been forthcoming in the examination of the blood in tubercular pleurisy. In spite of the fact that a lymphocytosis exists from the earliest stages in the exudation, no increase of the lymphocytes in the blood is seen. In every chemotactic increase the rule is that increase in numbers of a certain species of cell finds a corresponding increase of the same cells in the blood.

It therefore follows from clinical and morphological examinations, and also from the results of investigations of inflammatory processes, that the lymphocytes do not stand in any correlation to the polynuclear leucocytes. The same result will be arrived at in a different way in the following chapter.

Erythropoesis and formation of myelocytes have within recent times been observed under pathological conditions in the lymphatic glands, just as they have been seen in the spleen. The analogy with the conditions obtaining in connection with the spleen is a perfect one. During the embryonal period the myeloid tissue at first claims a place in the lymphatic glands as well, and only disappear gradually as the bone marrow develops. In post-embryonal periods the central portions of the glands undergo a myeloid transformation, in severe anæmias, in infections, and intoxication. This takes place more especially when the organism calls forth new fields for the production of the vitally essential red blood corpuscles and myeloid leucocytes, in compensation for defective function of bone marrow.

(c) The Bone Marrow

It was formerly thought that the spleen and lymphatic glands were the only organs of production of the blood corpuscles, but general attention was attracted to the bone marrow by the investigations of Neumann and a little later of Bizzozero, in which it was shown that the precursors of the red blood corpuscles are formed in these organs. This discovery was rapidly recognised, and was soon turned to practical use in pathology by Cohnheim and others. In this connection, especially valuable information was adduced in the fact that after severe hæmorrhage the medulla of the long bones was reconverted into red marrow, which shows that the regenerative function of the bone marrow may meet an increased demand.

No other site of production of red blood corpuscles in man under normal conditions is known. In other mammalians, as has already been mentioned (see p. 115), the spleen may participate to a certain extent in the production of erythrocytes. The type according to which the normal production of blood is carried out in adults, and the variations from this type which are met with in pernicious anæmia, have been discussed in detail in the chapter on the red blood corpuscles, and Ehrlich's views were accorded their proper significance, according to which the production of blood in Biermer's anæmia follows quite a different type, and one which is analogous to the embryonal type.

It is therefore only necessary in this chapter to consider the white blood corpuscles and their relation to the bone marrow. In man, as well as in a number of other animals (*e.g.* monkey, guinea-pig, rabbit, pigeon, etc.), the bone marrow shows a peculiarity in that the cells which it produces contain specific and easily demonstrable granulations. This is sharply contrasted to the lymphatic system, the granules of which are quite different, and which differ among themselves. Some of the latter, such as the azure granules and Schridde's fuchsinophile granules, were not recognised for a very long time.

The azure granules are only found in some of the lymphocytes, and possess quite a different biological significance to the acidophile, eosinophile, and mast cell granules. As Pappenheim has pointed out, the azure reaction of the granulations is not specific and may occur even in thrombocytes of frogs and in carcinoma cells.

A very important classification into two groups can be recognised in the granulated cells of the bone marrow.

The first group of the "special granules" claims especial consideration, because they are the characteristic sign of certain animal species. They show varying tinctorial and morphological behaviour according to the species of animal. For example, they have a neutrophile granulation in man and in the monkey; they have what Kurloff described as a pseudo-eosinophile granulation in the guinea-pig and rabbit; in birds two specific forms of granulation exist side by side. Both of these are oxyphile, and while the one occurs in crystal form, the other is deposited as granules in the protoplasm. The forms of special granules which have been studied hitherto have one characteristic in common, namely, that they stain with acid or neutral dyes, but show a much smaller affinity to the dye bases. The fact that the number of these granules far exceeds that of any of the other elements of the bone marrow shows how important they are.

The second group of the bone marrow cells contains granules which are found in the whole series of vertebrate animals, from the frog up to man, and which are therefore not characteristic of any one species. These are: (1) the eosinophile cells, and (2) the basophile mast cells.

The mononuclear cells represent the non-granulated cells of bone marrow. Under normal conditions they are less abundant and probably less important than the granulated cells, and especially than the predominating first group of granulated cells. Under pathological condition, however, this is not so.

Of the non-granulated cells, the giant cells deserve special mention, because they are an almost constant component of bone marrow in mammals.

It has been shown by the study of embryology and histology that the giant cells of bone marrow parenchyma are intimately connected with the myeloid tissue, and are usually met with whenever pathological conditions lead to fresh myeloid formation of cells.

When a stained dry preparation of bone marrow of the guinea-pig, rabbit, or man is examined it will be seen that characteristic finely granulated cells are present in all stages of development, from the mononuclear cells, through the transition forms to the polymorpho-nuclear cells, just as is the case in circulating blood. A glance at such specimens proves that the bone marrow is the incubator in which typical polynuclear cells are constantly being formed from the granulated mononuclear cells.

The same method of maturing can also be observed with regard to the polynuclear eosinophile leucocytes.

Ehrlich was able by means of special differential staining to supply evidence of the fact that during the transformation of the mononuclear cells to the polynuclear the kind of granulation also changes. In the young granules the basophile type is predominant, but as the cells mature this type becomes less marked. Thus the pseudo-eosinophile granules of the mononuclear cells of the guinea-pig stain bluish red, after prolonged fixation in superheated steam and staining with eosin-methylene-blue; in the transition forms this blue tone gradually becomes weaker, until at last the granules of the polynuclear leucocytes stain pure red without any blue admixture. Similar observations may be made with the eosinophile cells of the human subject and of animals, and with the neutrophile cells of the human subject. It has therefore become possible to decide whether a single granule belongs to a young or to an old cell.

It is at present unknown how rapidly the process of maturing of the mononuclear cells into polynuclears is completed, or whether the granules mature at the same rate as the cell as a whole. Nevertheless, the author is of opinion that some observations go to show that both these processes take place

simultaneously, and that in special cases the morphological ripening of the cells proceeds at a more rapid pace than that of the granules. It is especially easy to collect evidence of this nature with eosinophile cells. As early as 1878, in his first publication, Ehrlich stated that, apart from the typical eosinophile granules, other single granules were frequently met with which showed a different tinctorial behaviour; for example, when stained with eosin-aurantia-nigrosin, they appeared nearly black, or when the eosin-methylene-blue mixture was used they took on a bluish red or even a pure blue colour. Ehrlich recognised these forms even at that time as young elements. The same well-marked differences are met with in leukæmia, in the circulating blood affecting both the neutrophile and the eosinophile groups. Ehrlich has repeatedly come across polynuclear eosinophile cells in leukæmic blood, in which the granules had to be regarded almost exclusively as young forms.[1]

Ehrlich regarded this phenomenon in leukæmic blood as an expression of a typical hastening of the morphological process of maturing, as compared with the slower development of the granules.

Only the mature forms of the specific granulated leucocytes which occur in bone marrow are found in normal blood, while the mononuclear and transition forms of the neutrophile group do not pass over into the circulation under normal conditions at all.

Since these cells are only found in bone marrow, and are never present under normal conditions either in the spleen or in the lymphatic glands, Ehrlich regarded the mononuclear neutrophile granulated cells as the most characteristic of bone marrow, and therefore named them κατ' ἐξοχήν, "myelocytes." Whenever myelocytes, no matter of what size, appear in the blood of adults

[1] This double staining of the eosinophile granules has been interpreted by many authors, *e.g.* Arnold, on the assumption that eosinophile and mast cell granules can occur simultaneously in one and the same cell. That this is not correct is shown by the fact that alleged "basophile" granulation of the eosinophile cells does not show the characteristic metachromasia of the mast cells when stained by the usual metachromic staining methods, and that when Giemsa is used the precursors take on a blue and not a mauve colour, as is the case in mast cells.

in considerable numbers a very severe disturbance of the leucocyte production may with certainty be assumed. It must, however, be pointed out that, even when moderate numbers are present, the diagnosis of leukæmia is not necessarily justified, since it does occur that even in curable affections, and especially in severe forms of anæmia, 10 per cent. and more myelocytes are present. This is, however, extremely rare.

In this connection an observation made by Morawitz is particularly instructive. In a case of necrotic tonsillitis a very severe anæmia had developed, and the blood of this patient showed an enormous quantity of normo- and megalo-blasts. The neutrophile myelocytes in the blood were found to correspond to 20 per cent. of 22,100 leucocytes per c.mm., and the eosinophile myelocytes at times to 1 per cent. Improvement took place after transfusion of blood, and the patient eventually recovered.

It is, however, generally true that high myelocyte values, especially if associated with a high total number of white blood corpuscles, must be regarded as the most important symptom of a myeloid leukæmia.

Precisely similar conditions hold good for the eosinophile cells. The mononuclear cells in this case also (known as eosinophile myelocytes) occur in large quantities almost exclusively in leukæmic blood. This find, which was first analysed by H. F. Müller, represents a less useful indication, since the chief mass of the foreign elements of the blood of myeloid leukæmia is made up to a large extent of Ehrlich's myelocytes.

These observations admit of very important conclusions with regard to the question of leucocytosis. When it is considered that the polynuclear neutrophile cells are only developed from and stored up in bone marrow, and that in ordinary leucocytosis only the polymorphs are increased in the blood, it becomes clear that leucocytosis is a pure function of bone marrow. Ehrlich has always insisted rigorously on this. It is on this basis alone that a leucocytosis, which frequently sets in with extraordinary suddenness (this is often observed in diseases as well as in experimental conditions) can be satisfactorily explained. Under

these circumstances, the space of time during which the development of the leucocytosis takes place, which may be limited to a few minutes, is far too short for a new formation of leucocytes to be possible. It thus appears that there must be a place where these cells are stored up ready for use, and prepared to wander forth in response to every suitable stimulus. This place is the bone marrow, and no other. In this situation the mononuclear elements mature gradually, to become polynuclear contractile cells, in which condition they obey every chemotactic stimulus by migrating and thus produce an acute leucocytosis.

The bone marrow thus fulfils, besides its other functions, the highly important task of a protective organ. It can overcome, with rapidity and energy, certain noxious agents which threaten the organism. This organ is at all times prepared to avert danger, by responding promptly to each and every call, and to send forth its agents to take up the fight at the invaded place; it may indeed be likened to a well-organised fire brigade.

It should be mentioned that the large mononuclear leucocytes and the transition forms of normal blood are involved to a certain extent in the increase of cells in ordinary leucocytosis; the same applies in myeloid leukæmia. The appearance of the former kind of cell, however, in leucocytosis is peculiar and is not at present fully understood. The reader is referred to K. Ziegler and Schlecht's recent work on the subject.

From the biological point of view there can be no doubt that the group of cells of the transition type, among which Ehrlich's large mononuclear cells must be included, actually belongs to the myeloid system and is developed in it.

There are no reasons either of a histological or of any other nature which would justify the assumption that these cells are formed in the spleen; and there is also no proof that they are formed in the lymphatic glands. An adventitial normal origin of the large mononuclear cells, such as Helly has attempted to substantiate, has certainly not been proved to exist. According to this theory, these cells are regarded as being of the same species as the lymphocytes, and are termed for this reason

leucocytoid lymphocytes. In the opinion of the author this theory has been definitely disproved by the more minute analytical methods, especially by the demonstration of neutrophilic granules.

A further argument in favour of the myeloid origin may be sought in the fact that large mononuclear cells have been found in increased numbers in bone marrow as well as in the blood. This has been demonstrated in the experiments of Nattan-Larier.

It is therefore possible, on the basis of the microscopical appearances, to conclude that the bone marrow is by far the most important blood-forming organ, and that the red discs and also the chief group of the white discs, *i.e.* the polynuclear neutrophile cells, are exclusively formed in this situation.

Insurmountable difficulties are placed in the way of experimental physiological examination of the functions of the bone marrow. It is absolutely impossible to eliminate the whole bone marrow, or even a considerable part of it, by means of operation. No value can be attached to the attempts to determine the function of the bone marrow by comparative counts of the arterial and venous blood in a chosen bone marrow area. J. P. Roietzky, working under the direction of Uskoff, has carried out counts of this kind in the dog, using the blood from the nutrient artery of the tibia and from the corresponding vein. He found that the number of white blood corpuscles in the vein was slightly increased, but that the absolute number of "young blood corpuscles" (Uskoff), *i.e.* lymphocytes, was considerably diminished, and at the same time that the number of "mature" cells, which correspond to a large extent to what are usually termed the polynuclear cells, was markedly increased. The following table shows his results :—

	Total Quantity.	Young Cells.	Mature Cells.	Old Cells.
Arterial blood	15,000	1950 (13·0 %)	840 (5·6 %)	12,210 (81·0 %)
Venous blood	16,400	656 (4·0 %)	2788 (17·0 %)	12,956 (79·0 %)

The indispensable hypothesis for the value of such comparative counts would be the assumption of a continuous function of the bone marrow, and Uskoff appears to make this assumption. If bone marrow can absorb the lymphocytes continuously to such a degree, it would be difficult to understand how the normal condition can be maintained in view of the extent of the bone marrow and the rapidity of the circulation. There is, however, every reason to suppose, on the contrary, that the bone marrow acts intermittently, since, as has been set forth in detail in the preceding pages, elements are constantly maturing in the bone marrow and that these elements only migrate at certain epochs in response to chemical stimuli. From this argument it would follow that but little can be expected from the results of experimental methods like those of Roietzky.

But apart from this, Roietzky's experiments lose all their value when it is realised that the tibia of the dog, which he utilised in his experiments, contains grey and not red marrow. Professor Schütz has shown that this is true for all breeds of dogs, and it is further well known that grey marrow does not exercise the least hæmatopoietic functions.

This example, therefore, may be taken as a strikingly instructive one, proving that experiments of this kind cannot yield any reliable results.

Clinical experience of cases, in which considerable portions of the bone marrow are replaced by other forms of tissue, supply far more important evidence of the functions of bone marrow.

The chief source of this evidence has been obtained from cases of carcinosis of bone marrow. Reference has already been made to such cases.

In this affection large portions of the bone marrow may be replaced by tumour masses; under these circumstances, however, the organism is capable of assisting itself, by giving rise to a development of myeloid tissue in the spleen, lymphatic glands, liver, etc., as in embryonal periods. But since this form of tissue is normally never found in the spleen and lymphatic glands, the pathological appearance of myeloid formation reveals

with special clearness which functions the bone marrow has to perform.

Bone marrow may be replaced by typical lymphatic tissue as well as by the tissue of malignant growths. As Neumann has shown, and as has since been generally acknowledged, this takes place in lymphatic leukæmia. In cases of this condition large areas of bone marrow are occupied, not by malignant tumour masses but by lymphatic tissue.

As a counterpart of this lymphatic metamorphosis of bone marrow the myeloid transformation of other blood-forming organs in myeloid leukæmia may be cited. These organs include more especially the lymphatic glands, and the transformation can be recognised by the presence of myelocytes, eosinophiles, and nucleated red blood corpuscles.

The substitution of myeloid tissue in lymphatic leukæmia is, it is true, seldom complete, and even when it is so, myeloid tissue will be seen to appear in other blood-forming organs. This is the explanation why the neutrophiles may always be found, even if it be in very small numbers.

The neutrophile elements disappear most rapidly in acute lymphatic leukæmia, because the abnormal proliferation takes place with great rapidity, and for this reason induces a quick and uncomplicated elimination of the tissue of the bone marrow, as if it had been provoked experimentally. The neutrophile elements of the marrow therefore disappear rapidly in these cases, and in some so completely that, as in Ehrlich's case, it may be difficult to find a single myelocyte. The fact that in this case an advanced absolute diminution of the polynuclear leucocytes in the blood was met with, is accounted for on the ground that these cells are derived from bone marrow, and consequently, if the bone marrow is destroyed, no more of these cells could pass over into the blood.

The lymphatic substitution of the marrow was more marked still in a case of chronic lymphæmia observed by the author, in which death followed rapidly as a result of sepsis supervening. The blood did not contain a single neutrophile cell among the

many thousand leucocytes, and in the organs also not one could be found.

A temporary myelocytosis of the blood takes place frequently in the early stages of lymphæmia as a result of stimulation of the bone marrow. But a continuous decrease in the numbers of the myelocytes in the blood can be seen later, keeping pace with the replacement of the tissue of the medulla.

The bone marrow has, as has recently been determined, another extremely important function besides that of forming cells. This function is the power of producing antitoxins (Wassermann, Pfeiffer, and Marx). It therefore must be regarded as the organ *par excellence* which has to decide the termination of an acute infection.

The red blood corpuscles are further disintegrated in the bone marrow, and the available material is utilised again for the reconstruction of new cells.

III.—ON THE DEMONSTRATION AND SIGNIFICANCE OF CELL GRANULES.

In recent times, histological, biological, and also clinical investigation has aimed, to an ever-increasing extent and with most promising results, at the solution of the problem of the significance of the cell granules. The work undertaken with this view has proved of great importance to hæmatology, and it is now clear that a number of important questions which have still to be answered are intimately associated with the study of granules. It would therefore appear to be advisable in this place to deal with the history, methods, and results obtained up to the present from the investigations concerning cell granules in a comprehensive manner.

The credit of having first pointed out the great importance of granules, and of having obtained practical results by systematic long-continued investigations of this subject, undoubtedly belongs to Ehrlich. It seems necessary to emphasise this fact, as Altmann has repeatedly maintained that it is not the case, in spite of the

fact that his attention has been called to the real state of affairs. After Ehrlich replied to Altmann's priority claim in a special article in 1891,[1] Altmann stated in the second edition of his *Elementarorganismen*, which was published in 1894, that he was the first to recognise the specific importance of the granules, and that although these bodies had been observed by a few authors, they had only been regarded as "specialities and isolated appearances." It is therefore necessary to quote a few pregnant passages from Ehrlich's work.

It is quite clear that Ehrlich did not regard the granules as "isolated appearances" in one of his earliest publications on this subject, which appeared in 1878, that is, ten years before Altmann's contributions. It must be admitted that an author who devotes ten years' work almost exclusively to a single subject could not but regard the subject of his investigations as of considerable biological importance.

In respect to this matter, Ehrlich wrote: "The word 'granulated' has been employed with predilection since the beginning of histology to indicate a constitution of cellular structures The choice of this expression is not a very happy one, since very many circumstances may lend the appearance of granulation to protoplasm. Modern methods of examination have shown that many elements which were described by earlier authors as granulated owe this impression to the presence of a reticulated superimposed protoplasmic network. These cells are just as little granulated as are cells which show granulated albumin precipitation resulting from cadaveric coagulation or from the influence of certain chemical reagents such as alcohol. The term should therefore be reserved for the elements, which include substances in granular form during life, that can be distinguished by chemical means from the normal albuminous substances of the cells. Only a few of these granulations, like fat and pigment, are easily recognisable; the majority cannot be characterised, or can only be indistinctly characterised with the help of the methods now in

[1] See Ehrlich, *Farbenanalytische Untersuchungen XII., zur Geschichte der Granula*, p. 134.

general use. It used to be considered sufficient to determine the presence of granules in certain cells, and according to whether they refracted light or not, to register them as either fat or albumin granules.

"Previous experience, especially in connection with mast cells, has induced me to expect that the characteristics of these granules, which have so long resisted chemical examination, might appear sufficiently sharp, by means of colour analysis, *i.e.*, by means of their behaviour to certain staining agents. As a matter of fact, I have found these forms of granules, which could be recognised by their elective attraction for certain dyes, and which can therefore be readily traced through series of animals and of organs. I have further been able to prove that certain kinds of granulation, which I have discovered, are only found in certain cellular elements. These granules characterise the cells in the same way as pigment characterises the pigment cells, and glycogen the cartilage cells (Neumann), etc. Just as the diagnosis of the mast cells, which show so many variations, can only be made from the granulation which stains with dahlia, *i.e.* from the result of a chemical reaction, other granulated cells, which cannot be distinguished from one another morphologically, can readily be classified into definite sub-groups on the basis of their tinctorial behaviour. In consideration of these differentiating characteristics I would propose to call such granulation 'specific granulations.'

"The examination was carried out by Koch's method of preparing very thin smears of the fluids (blood) or the parenchyma of the organs (bone marrow, spleen, etc.) on cover-slips, allowing these smears to dry at room temperature, and then staining them after varying intervals. I chose this apparently somewhat rough method more especially because, for the histological detection of new granules which may possibly correspond to definite chemical compounds, it was necessary to avoid using any substances which, like water or alcohol, might act as solvents or, like osmic acid, etc., as oxydising agents. To gain this end, only methods of procedure like simple drying would preserve the chemical individuality as little changed as possible."

Further advance in this extremely complicated section of histology, however, was only rendered possible by an exact study of staining processes, and of the relations which exist between chemical constitution and staining characteristics. The first result of this investigation which no one had ever undertaken before was the sharp definition of acid, basic and neutral dyes, and of the corresponding oxy-, baso-, and neutrophile granules. It was only possible after experimenting with many hundred combinations to discover the triacid solution, which has played a highly valuable rôle in the demonstration of the most important phenomena.

The classification of the cell granules in the blood, constructed with the assistance of these methods according to their varying chemical affinities, is still accepted as the best and indeed as the only useful method of grouping the leucocytes. Ehrlich laid special stress from the first on the fact that various forms of cells included various forms of granules, which could be distinguished from one another, not only by their tinctorial behaviour, but also by the special way in which they responded to various solvents.

Altmann's method, which depends on a complicated fixation process and a single unvarying method of staining, must be regarded as retrograde from this point of view, since it is likely to obscure the principle of the specific character of each type of granulation.

Another disadvantage of Altmann's method of hardening consists in the fact that it precipitates albuminous substances included in the cells in the form of round grains which take on stain like real granules. In this way it becomes extremely difficult to distinguish preformed from artificial elements. Since A. Fischer's publication, in which he demonstrated the experimental production of granule-like artefacts by various reagents, many authors have recognised that there are grave doubts as to the reality of Altmann's granules. In contrast to this, Ehrlich's dry method has been shown to be quite free from objection. Granules cannot be produced artificially by drying, and what is seen in the stained specimens corresponds exactly to what is seen in fresh living

blood. The chief value, however, of the dry method is, that the chemical individuality of the various granules remains quite unaltered, so that all the chemical differential experiments take place in an object which is practically intact.[1]

A further manner of gaining an insight into the nature of the granules depends on the principle of vital staining. The first attempt to stain granules in living animals was stimulated by Ehrlich's vital methylene-blue staining, the practical importance of which to neurology has been universally recognised. One of the earliest publications on this subject was that of O. Schultze, who immersed frog larvæ in dilute methylene-blue solutions and after a short time found a blue staining of the granules, especially of the intestines, of the red blood corpuscles and of other kinds of cells. This method, however, as Ehrlich has experienced on many occasions in his methylene-blue studies, is not quite free from objection, since methylene-blue, after it has been used for some time, frequently forms granular precipitates which can be confused with granules. Teichmann deals with this point in an exhaustive discussion, and considered that the majority of the granules which Schultze described are artificial productions.

Neutral red, which was first recommended for this purpose by Ehrlich, and which has since been employed successfully by Przesmycki, Prowazek, S. Mayer, Pappenheim, and others, is highly suitable for the study of vital staining of granules. This dye was compounded by O. N. Witt out of nitroso-dimethyl-anilin and meta-toluylen-diamin. It is the chloride of the basic dye, and is soluble in pure water, forming a fuchsin-coloured solution. In solution in weak alkaline fluid (the alkalinity of spring water is sufficient for this purpose) the dye takes on a yellowish-orange colour.

Neutral red has a special characteristic in that it possesses an almost maximal affinity for the majority of granules. Ehrlich was able to demonstrate the presence of granules even in some

[1] Altmann's freezing process would correspond to the conditions which Ehrlich has always insisted upon. But it presents such great technical difficulties that up to the present it has not found acceptance.

plant cells by means of this dye. The method of application is extremely simple. In the higher animals numerous granules can be stained by subcutaneous or intravenous injection or even by feeding. Frog larvæ and mollusca may be sufficiently stained, if they are allowed to swim about in dilute solutions of the dye. Even in "surviving" (*überlebende*) organs the staining may be successful. It is best carried out by allowing small portions to float about for a time in physiological salt solution to which a trace of neutral red has been added, with free access of air. As soon as the object appears red to the naked eye it is ready for examination.

As might have been expected, the most beautiful results are obtained with organs which can be easily teased out, such as the eggs of flies, the Malpighian canals of insects, etc. The solution of the stain should be so prepared that the act of staining does not take too long, but, on the other hand, that the concentration of the dye is not so high that the nucleus of the cell takes any of it up. One part in 50,000 to one part in 100,000 suffices as a rule for this purpose. The hæmatologist tries to prepare films in which only the granules of the cell are stained, while the protoplasm and nucleus appear unstained. Artificial productions cannot be entirely excluded even with this method. In cells of plants which contain tannin the production and precipitation of tannates of the dye may simulate granules. It is, however, not difficult for an experienced observer to recognise these artificial products as such. The kind of granulation, their typical distribution, a comparison with neighbouring cells, the combination of various methods, a comparison of the same object stained with vital staining and with "surviving" staining, all contribute toward making it easy to form a proper opinion with regard to the granules, and to protect the observer from making mistakes.

The majority of the granules in vertebrates are stained orange red, in accordance with the mildly alkaline condition of these structures. Grains which stain a pure fuchsin colour are met with much less frequently, and these must accordingly have a weak acid reaction.

Combined methods of staining can be used as valuable auxiliaries to neutral red staining. Ehrlich employed a double staining with neutral red and methylene-blue, by immersing frog's larva in a solution of neutral red to which a trace of methylene-blue had been added. He found the granulation almost exclusively red, but that of unstriped intestinal muscle was stained an intense blue. He further obtained a still more marked differentiation of living cell granules by means of a triple combination. There is no doubt that a careful study of the neutral red methods will bring to light further important details in respect to the nature and function of granules, and will illuminate some of the most delicate problems of cell life. The knowledge which has already been acquired admits of definite conceptions of the biological significance of the cell granules.

A number of interesting finds have been recorded during recent times as a result of studies with vital staining by Arnold, Rosin and Bibergeil, Pappenheim, and others.

Ehrlich described the granules as metabolic products of the cells in his first publication. He believed that these products were deposited in solid form in the protoplasm, to serve partly as reserve material and partly to be cast out of the cell. He only departed from this view for a short time when he came to consider the observations made with liver cells, which have been exhaustively described in Frerich's well-known work (1883, p. 43). Ehrlich showed that the liver cells in dry specimens of rabbit's liver, which is rich in glycogen, appear as voluminous, polygonal elements with symmetrical, homogeneous brown colour, limited externally by a narrow, sharply defined pure yellow membrane. It could be seen that the cells which did not contain much glycogen, contained in the homogeneous glycogen-brown content small rounded pure yellow particles which were obviously of a protoplasmic nature. "The application of dyes revealed that the hyaline, glycogen-carrying substance filling the cell was not stainable under any circumstances, while the membrane and the granules, which are present in the cells,

stain readily with almost any dye. It was further possible to demonstrate, by means of dyes, that the membrane is chemically different from the granules, since when eosin-aurantia-indulin-glycerine is employed, the membrane colours blackish and the granules a reddish orange."

From these observations Ehrlich deduced the following conclusions, which may be quoted literally: "That the cells of the liver during the period of feeding contain a narrow protoplasmic membrane and a homogeneous content which carries glycogen, and in which the nucleus and the round granules of (functionating) protoplasm are embedded.

When these results are compared with the knowledge which has been gained within recent times with regard to cells it becomes easy to determine the exact position where the glycogen is deposited. Kupffer first established the fact with regard to liver cells, that the contents of the cell do not represent a uniform body microscopically. This has proved to be true for all cells. In the "surviving" object, two distinctly different substances are found beside the nucleus; the one, a hyaline matrix, which constitutes the greater part of the mass, and the other, a sparse finely granulated fibrillary substance, which is embedded in the former. Kupffer called the former paraplasm and the latter protoplasm. By warming the objects to about 22° C. a distinct but slight movement was observed in the network. There can be no doubt but that of these two substances, the granulated reticulated one—the protoplasm—is the more important. It may be assumed that the granulation of the network is the centre of the real specific function of the cell. Any way, it will be advisable to give a special name, such as microsomes, to these structures, which in the liver cell form round or oval granules staining yellow with iodine, and taking on other dyes easily and intensely (Hanstein)."

It was necessary to quote this old work extensively to show that Ehrlich had, even as long ago as 1883, described the granules as the actual carriers of the function of the cell; this view was enunciated many years later by Altmann under the

name "bioblast theory." Consequently the foregoing may be considered to be ample evidence that Altmann's repeated claim, that no one had accorded a full significance to the granules prior to him, is absolutely untenable.

The following quotation from Altmann's work (*Die Elementarorganismen*, 1st Ed., p. 39) shows what significance he ascribed to those granules which he termed "ozonophores."

"The term ozonophore is intended to convey the special idea, which is calculated to take the place of the older conception of living protoplasm, at all events as far as its vegetative function is concerned, and which is capable of serving as a basis for the varied processes of organic metabolism. To recapitulate the capabilities of the ozonophores, it may be said that they perform reducing as well as oxydising functions, by the transport of oxygen, and thus cause the disintegration and synthetic construction of bodies, without losing their own individuality in the least degree."

In the meantime Ehrlich has made several observations which cannot be reconciled with his own earlier hypotheses, and with Altmann's far-reaching deductions. His investigations with regard to the oxygen requirements of the organism had taught him that "ozonophores" cannot possibly be integral constituents of the cells. The fact that there are normal cells in which no granules can be detected by any of the ordinary methods had also to be taken into account. Finally, a pathological observation showed that it is impossible to defend the view that the granules are the carriers of the cell function. It has been shown in fishes and in other lower animals that the granules can be made to disappear experimentally by starvation. In the examination of a case of pernicious anæmia (see *Farbenanalytische Untersuchungen*) Ehrlich found that the polynuclear cells of the blood and of the bone marrow, and also their precursors, were free from neutrophile granulation. This find caused him to return to his older view, that the granules were the secretion products of the cells, and he expressed his opinion in the following words:—

"If the neutrophile granules were really, as Altmann supposes, elements which supply the cell with oxygen, the condition which I have just described would be excluded, since the disappearance of the granules would produce the death of the cell. The conditions described, however, can easily be explained on the basis of the secretion theory, just as this theory could explain how a fat cell can lose its contents completely without dying. The same applies to the bone marrow cell when the blood fails to supply the necessary precursors, under which circumstances it cannot form any further neutrophile granules, and must therefore be transformed into non-granulated cells."

The great chemical differences between the various forms of granules may be regarded as evidence in favour of the view that the granules are actually the metabolic products of the specific activity of the cells. Ehrlich has paid special attention to these conditions in the blood, and has found that the granules in the blood cells not only differ from one another with regard to staining reactions, but also with regard to shape and solubility. It was therefore necessary to draw sharp lines of demarcation between the various kinds.

It can be shown that while the majority of the granules are more or less round structures, in some animal species, *e.g.* in birds, analogies to the granules of the mammal's blood are met with which are characterised by a well-marked crystal form and decided oxyphilic qualities. The substance contained in the mast cell granules occurs in some species of animal in a pure crystalline form.

The size of the individual grains in the blood cells of each species of animal is a definite one for each kind of specific granulation. The mast cells alone form an exception to this rule. The eosinophile granules, for example, reach their maximum in the horse, where they assume giant proportions.

The occurrence of granulated colourless blood cells has been demonstrated in practically every species of animal. This has been emphasised by Knoll, who found that it obtained even in the case of many invertebrates, more especially in the

lamellibranchiates, polychætes, pedates, tunicates, and cephalopods.

Exact and numerous examinations in this regard have been conducted with the blood of the vertebrates, and especially the higher forms. For example, two forms of oxyphile granulation are known in birds, of which one kind of granule is embedded in the cell in crystal form and the other in the ordinary grain form. The majority of the mammals whose blood has been examined possesses granulated polynuclear cells. Hirschfeld has dealt with this subject in an exhaustive work, in which a large number of very remarkable details are contained. He found that in the majority of the animals examined the polynuclear cells were provided with neutrophile granules, and only in one animal, the white mouse, did he fail to find this or an analogous form of granulation.

The statements made by Hirschfeld cannot be accepted, in view of some investigations undertaken by Dr. Franz Müller in Ehrlich's laboratory. After many fruitless attempts Dr. Müller discovered a method by means of which he was able to demonstrate numerous but extremely fine granules in the polynuclear cells of the mouse. This shows that it is not permissible to assume the absence of granules, even if the ordinary staining methods do not suffice to reveal any. Just as there is no universal method of demonstrating bacteria, so there is none for rendering granules visible. All the granules which consist of soluble substances must necessarily disappear when the ordinary triacid method is employed, thus simulating a homogeneous cell protoplasm.

The foregoing, however, is not intended to indicate that the occurrence of non-granulated polynuclear cells is denied in certain animal species. Hirschfeld states that such cells exist side by side with granulated cells—for example, in the dog, and from this find he deduces far-reaching conclusions with regard to the significance of the granules. It must, however, be pointed out that Kurloff's work tends to show that there is no reason for assuming that the non-granulated polynuclear cells are

identical with the granulated cells. Kurloff was able, at all events as far as the blood of the guinea-pig is concerned, to prove that these two different elements may be sharply distinguished from one another and that they each have a separate genesis.

The fact that in general only those cells of the blood which are meant for migration and chemotaxis, and which are capable of carrying out these functions, contain granules must be regarded as highly important. This applies to all species of animals. It is a very suggestive assumption, which can scarcely be disproved, that the migration of the granulated cells has a certain degree of nutritive character, and for this purpose just those cells which enclose abundant quantities of reserve material would be peculiarly adapted. On the other hand, the lymphocytes do not contain the kind of granulation met with in the myeloid cells, nor are they involved in the chemotactic process.

A further indication that the granulation actually is connected with a specific activity of the cells is found in the fact that one cell is the carrier of only one specific kind of granule. Ehrlich was able, on the basis of investigations undertaken specially to clear up this point, to show that the contrary opinion, which recognised the simultaneous occurrence of neutrophile and eosinophile granulation or of eosinophile and mast cell granulation in one and the same cell, was not in correspondence with fact. This contention has been fully confirmed during the past ten years in an almost innumerable series of control experiments. The author, who has carried out an extraordinarily large number of examinations has never met with the combination in question even when the most severe pathological conditions have existed, either in the blood or in the blood-forming organs. Ehrlich has never observed the alleged transformation of the pseudo-eosinophile cell of the rabbit into the true eosinophile cell.[1]

[1] The cause of this kind of misunderstanding is to be sought in the developmental stages of the granules, when the tinctorial characters show variations, as has been described at some length on a preceding page. How little tinctorial variations alone suffice to determine the chemical identity of granules becomes quite clear when the granules of other organs are taken into consideration. No one would

With regard to the transformation in the rabbit, it may be stated that the best method of proving that this does not take place is by utilising the fact that the different granules behave differently towards the various solvents. For example, the pseudo-eosinophile granules can be completely extracted from the cells by acids, while the eosinophile granules are left intact by this procedure and can then be stained alone.

The most convincing proof that the neutrophile, eosinophile, and mast cells are absolutely differentiated from one another by the original differences of the protoplasm, of which the granulation is but one, albeit a peculiarly striking, character, is found in the study of the various forms of leucocytes. As will be proved in detail in the following chapter, the neutrophile and the eosinophile leucocytes behave quite differently as regards their chemotactic susceptibility to stimulation. Those substances which call forth either a positive or a negative energetic chemotaxis in one group of cells fail to exert any influence on another group. At times even an opposite effect is noted, in that a substance produces an attraction for one kind of cell and a repulsion for another kind. The behaviour of the mast cells in this respect shows a still more striking difference. As far as the subject has been investigated, those substances which exert a chemotactic effect on the neutrophile or eosinophile cells do not influence the mast cells at all.

In accordance with the characters of the granules as specific cell secretions, the various kinds ought to be differentiable in so far as their chemical peculiarities are concerned. The granules of the blood corpuscles appear to possess a relatively simple chemical composition. There is reason to believe that the

ever dream of asserting that a liver, muscle, or brain cell could secrete pancreatin simply because the granules of the pancreas as demonstrated by the various staining methods showed the same staining characteristics as those of the above-mentioned cells. The author wishes to state most emphatically that he is only prepared to recognise a uniform character of each kind of granulation to the full extent when this applies to the blood cells, in which the granules have a comparatively simple function, and that the highly complicated glandular cells, which must perform several functions simultaneously, may include several kinds of granules.

crystalline granules consist chiefly of one single chemical compound, which need not even be highly organised and which, like guanin, fat, melanin, etc., seems to be a relatively simple substance. The other forms of granules no doubt have a more complex composition and are probably mixtures of chemically separate substances. The most complicated granules of the blood are the eosinophilic, which, as has already been pointed out, possess a higher histological structure, including a peripheral layer which can be distinctly distinguished from the central portion of the granule.

It is quite probable that the granules are gradually given off to the neighbouring tissue. It is true that proof of this is extremely difficult to produce, and much of what has in the past been regarded as evidence for this elimination has turned out to be erroneous. An example of this is the so-called areola of the mast cells. In the case of the mast cell areola, it is fairly evident that in the specimens the granulations which are extremely soluble in water were not sufficiently fixed.

On the other hand, it is by no means difficult to demonstrate in old pus a rarefication of the polymorpho-nuclear neutrophile granules, which may be almost complete. All other explanations, save that of the casting off of the granules into the surrounding tissue, would appear to be unsatisfactory in this case.

IV.—THE DUALISTIC DOCTRINE

It may be said that at the present time there is no longer any really earnest opposition to Ehrlich's doctrine of the specificity of the granulations and of the formed mature types of leucocytes. This doctrine may therefore be recorded as a definite fact. But an energetic struggle still exists with regard to the further question, as to whether a sharp division should be drawn between the lymphatic and the myeloid systems and between the cells which are derived from these two tissues. Ehrlich has enunciated this dualistic doctrine as the most important result of his long-continued studies. A large number of opponents have disputed the correctness of this view, among

whom Arnold, Neumann, May, Grawitz, Maximow, Weidenreich, Hirschfeld, and Pappenheim may be named; while the most energetic supporters of his teaching are Banti, Türk, Sternberg, Helly, Schridde, Erich Meyer, K. Ziegler, Naegeli, and others.

The disputed question may be expressed as follows: Can, under given conditions, myeloid-tissue formation as well as lymphatic formations arise post-embryonally from lymphocytes? Some opponents of the dualistic doctrine maintain that the ordinary mature blood lymphocytes possess the capability of transforming themselves at will into any other form of cell (Grawitz). This view, however, is no longer tenable.

The possibility that young still immature organ cells having the histological characters of lymphocytes may take on a different development in the blood-forming organs is a more reasonable one. The supporters of this view regard the non-granulated cells of the myeloid tissue without further ado as lymphocytes; but in the opinion of the author, there are convincing arguments against such an assumption, as has already been stated.

If all the arguments for and against the dualistic doctrine be reviewed it must be admitted that the past few years have brought some very important facts which speak in favour of this doctrine. The embryological, histological, biological, and clinical experience in particular have forced the hæmatologist to a very definite conclusion which can only be expressed as follows: Ehrlich's dualism—the ingenious idea of the creator of hæmatology, has been definitely proved as correct. Those opponents who refuse to agree with this conclusion must be prepared to be reproached with a limited knowledge of histology, and with the statement that they have not studied the whole problem with sufficiently fine histological methods, *e.g.* section staining, and that they have not penetrated deeply enough into the cytological aspect of the question. The most solid support of medical science—pathological anatomy—has spoken the decisive word, but not, it is true, until a most intricate technique had been requisitioned.

The author has been able to show by embryological investigations that the myeloid system first develops, and that much later, and as an absolutely separate phenomenon, the lymphatic system follows. In view of this find, the idea which had been expressed over and over again, that myeloid tissue is only a more highly developed form of lymphatic tissue, must fall to the ground.

What do the opponents of the dualistic doctrine say to meet this argument? They maintain, without producing any histological evidence, that lymphatic tissue is more highly developed myeloid tissue!

The finer histological researches have shown that in adult life these two systems never transcend from one to the other, but that they actually stand in opposition to one another. Under no circumstances has it been proved that the germinal centre, for example, of the lymph follicles can act as the site of origin of myeloid cells. More than this, E. Meyer and Heineke, Naegeli, Ziegler, Schridde, and others have observed that myeloid proliferation always appears independently, and perhaps adventitiously, replacing the lymphatic tissue and gradually substituting it. Transformation never takes place, but only replacement and destruction. In this way the myeloid proliferation in the spleen first induces a diminution in size of the Malpighian bodies, and then causes them to disappear altogether; while in lymphatic proliferation in the bone marrow a lymphatic tissue springs up around the vessels and embraces them closely, isolating the areas of normal medullary tissue and finally destroying them.

In delicately stained sections it can be seen that, under normal conditions, lymphocytes are only to be found in scanty numbers in the sheaths of the vessels and never in the parenchyma. This is especially well shown in specimens stained by Schridde-Altmann's method, which characterises every lymphocyte most definitely.

The cells derived from the two tissues show an absolutely different biological activity. Only those derived from the

bone marrow show real chemotaxis. An increase in the numbers of lymphocytes in an exudation must depend on a local condition, since a large number of these cells are not present in the blood. The cells of these two tissues behave quite differently in disease conditions, so that absolutely striking differences may be noted in the blood appearances (cf. the blood curves of enteric fever, pneumonia, variola, etc.).

The myeloid cells contain oxydising and autolytic peptic ferments (the former is evidenced by the guaiacol reaction), but these are never found in collections of lymphocytes or in organs possessing an exclusively lymphatic nature, such as normal lymphatic gland.

The structure of the two forms of tissue is absolutely different. In bone marrow a loose tissue with irregular intermixture of various kinds of cells exists, while in the lymphatic system a regularly arranged structure is seen, in which follicles and germinating centres are situated.

These are the most important arguments which impel the hæmatologist to accept the dualistic doctrine.

There still remains one question to be answered, namely, how is it possible for the so-called metaplasia to occur under certain circumstances; *e.g.*, when myeloid formation makes its reappearance in the lymphatic glands and in the spleen? Some authors believe that this is due to a deposition of cells from the blood channels (Ehrlich, K. Ziegler). It seems, however, that there is reason to believe that it is due to local reactions, because the new tissue arises around the vessels, while in a number of observations no increase of myelocytes has been found in the blood.

We are thus faced with one of the most difficult problems at the present time. Can new formations arise out of indifferent adventitia cells or out of the "cells of the vascular wall" (Schridde)?

The following views appear to be calculated to throw the greatest amount of light on the subject at present, and are those which are most frequently brought forward in discussion.

1. According to Marchand, those cells which are associated with the *tunica adventitia* of the vessels are capable of participating in a myeloid transformation. The author supports this view warmly. It is quite easy to demonstrate histologically the adventitial position of myeloid metaplasia, not only in the embryo but also for the conditions obtaining in the adult. These myelocyte depots are often seen in the neighbourhood of even large vessels. It can be assumed that the cells implicated are indifferent cells which have retained their embryonal character, according to general biological laws (cf. Eugen Schultz), and not cells which have already passed through a stage of differentiation and specialisation.

One of the most difficult questions to decide is whether these indifferent cells may be transformed into myeloid or lymphatic tissue, according to the type of stimulus, or whether there are two different kinds of indifferent cells in the adventitial coat, the one of which is destined to become lymphatic and the other myeloid. There can be no doubt as to the formation of lymphatic adventitial depots. Extensive examples of this are seen in caseous pneumonia, without a single lymphocyte or any considerable number of plasma cells being present in the blood.

The author, working with H. Fischer, has recently been able to show that during the developmental period erythropoiesis and myelopoiesis occur independent of the *Tunica adventitia* in young embryonal connective tissue, far away from any vessel. It therefore seems probable that connective tissue cells which have retained their embryonal characters may develop into myelocytes as well as adventitial cells.

2. On the other hand, Schridde has enunciated the view, that in the earliest embryonal period the "cells of the vessel walls" give rise to the new formation. He speaks of this as heteroplasia. In post-fœtal myeloid metaplasia he suggested that similar cells, which had retained their embryonal characters, serve this purpose. He does not exclude the possibility of an adventitial genesis, in the sense in which Marchand and Naegeli

use the term; he is, however, inclined to regard the cells as being cells detached from the vascular wall, and deposited in the adventitia.

The theories in vogue at present depend on the assumption of the presence of cells which have retained embryonal characters and possibilities of development. This assumption is justified in the light of our present-day knowledge, and is supported by the studies of other organs (*e.g.* œsophagus—Schridde).

There is, however, one other possibility. Certain conditions are becoming known, in which differentiated cells lose their differentiation and return to their embryonal types. These cells can become differentiated again from their simple condition in a new direction. The reader is referred to the highly important details given in Eugen Schultz's work on reversible developmental processes, in which a number of convincing facts with regard to the animal and vegetable kingdom are recorded. This "undifferentiation" or "dedifferentiation" (Schultz) has played a considerable part in hæmatological literature during recent times (see Naegeli's text-book). It has, however, not yet been possible to produce actual proof of the occurrence of this process.

Schridde has assumed recently that, apart from the theory of the preservation of embryonal cells of the vessel wall, endothelial cells may become undifferentiated (indirect metaplasia), and thus produce myeloid tissue. This subject opens out great possibilities for future research.

V.—LEUCOCYTOSIS

The problem of leucocytosis has been subjected to as much discussion as any question of modern medicine. An exhaustive recital of the work devoted to it, of its methods, and of the results of this work would fill a whole volume, and would be out of place in a treatise on blood diseases. It is therefore only possible to describe the most salient points in connection with this subject in general terms. Only the purely hæmato-

logical aspects of the question will be dealt with in detail.

Virchow gave the name leucocytosis to a temporary increase in the number of leucocytes in blood, and taught that this occurred in a very large number of physiological and pathological conditions. During the period following the introduction of the term special attention was paid to the occurrence of leucocytosis in infective diseases, and the researches of the last fifteen years dealing with this subject have brought to light some very important information with regard to the biological significance of this phenomenon. The name of Metchnikoff must be mentioned in the first place with regard to this matter. This investigator was able to revolutionise our ideas by means of his phagocyte theory. Even if this theory has not been able to withstand criticism in many salient points, it has certainly stimulated work on this subject, and has been fruitful in advancing our knowledge of it.

In order to sketch Metchnikoff's doctrine briefly, it is necessary to transcribe the very suggestive word, phagocyte—scavengers. By this term is meant that the leucocytes protect the organism against harmful micro-organisms by catching them up in their pseudopods, by investing them and thus robbing them of the possibility of exerting their deleterious action externally. The termination of an infective process would therefore depend alone on whether leucocytes possessing this function are present in the blood in sufficient numbers to overcome the invasion of the germs.

In spite of its very plausible nature, Metchnikoff's doctrine has been markedly limited by further investigations. Denys, Buchner, Martin Hahn, Goldscheider and Jacob, Löwy and Richter, and many others have proved in numerous publications that the most powerful weapons of the leucocytes are not the mechanical pseudopods, but that their chemical products (alexins of Buchner) yield the strongest protection to the organism. The leucocytes are able, by means of the bactericidal or antitoxic substances which they give off, to paralyse the toxins

produced by the bacteria, and in this way they render the microbes harmless by depriving them of their weapons of attack, even if they cannot destroy them.[1]

The explanation of the fact that in bacterial diseases leucocytes are present in the blood almost always in increased numbers, is based on the principle which was discovered by Pfeffer of chemotaxis. This is equally applicable whether the chemical or the phagocytic doctrine of leucocytosis be accepted. According to this principle, bacteria or their products are able to attract the cells stored up in the blood-forming organs by means of chemical stimuli (positive chemotaxis).

This, however, by no means offers a satisfactory biological explanation for leucocytosis. It could be shown that conditions exist, like croupous pneumonia without leucocytosis, in which, in spite of the presence of chemotactically active substances, no increase of white blood corpuscles appears in the peripheral blood.

Bauer was able to present a particularly instructive example of this. He showed that an injection of the oil of turpentine failed to produce leucocytosis in a case of enteric fever as it does under ordinary conditions. The number of leucocytes present in the blood in enteric fever is not altered. After the patient has lost his fever and the medical practitioner has almost forgotten about the injection, the localisation abscess which he had tried to produce then appears. The explanation is quite obvious. Leucocytosis does not merely depend on a mere

[1] Naegeli appears to have somewhat confused the issues by classifying bactericidal and antitoxic properties together. With regard to the latter, it must be remembered that hitherto a comparatively small number of these substances has been found in the serum of animals suffering from bacterial diseases. With regard to the bactericidal properties, Naegeli appears to have overlooked that the very name indicates a killing of the bacteria, and for this reason the possible protection afforded to the organism would depend in this case on the removal of the living germs themselves. Whether this bactericidal action and the bacteriolytic action, with which it is closely associated, are free from further effects than those of protection cannot be discussed in this place. It must further be pointed out, that while the leucocytes yield ferments, usually known as alexins and complements, the action of killing or dissolving bacteria or of paralysing their products should not be regarded as properties of the leucocytes.—(The Translator.)

chemical attraction of cells from the blood and bone marrow: it depends first and foremost on a stimulation to increase the function of the bone marrow, and only as a secondary process does the chemotaxis come into play. If the bone marrow is incapable of responding to the stimulation by increased activity no leucocytosis takes place, in spite of the fact that the chemotactic substances are present.

It used to be taught that in those diseases which were characterised by a diminution of leucocytes in the blood, *i.e.* by leucopenia, there was a negative chemotaxis, and that the cells are repulsed by chemical substances produced in these diseases. Leucopenia is, as a matter of fact, a much more complicated biological process, and its origin in a negative chemotaxis is extremely doubtful

Numerous phenomena have been elicited in connection with the careful study of leucocytosis produced experimentally by bacterial toxins and proteins, organ extracts, poisons, etc., which still need special explanation.

Löwit was able to show that when these substances were introduced, two separate stages could be distinguished in the behaviour of the leucocytes. First, there was a stage in which the leucocytes were diminished in number (leucopenia—Löwit). In this stage only the polynuclear cells were diminished in number, while the lymphocytes were present in their normal proportions. Following this came the phase of increase of the white corpuscles, which also only affected the polynuclear cells: polynuclear leucocytosis. This behaviour seemed to indicate that the first period signalised a destruction of white cells by the agency of a foreign substance, and that the dissolved products of the leucocytes chemotactically produced a migration of fresh leucocytes. This view, however, soon met with various objections. Goldscheider and Jacob demonstrated, by means of painstaking experiments, that the temporary leucopenia of the blood was not a true one, but was only apparent, and was caused by an alteration in the distribution of the white blood corpuscles inside the vascular system. While the blood of the peripheral

vessels, from which as a rule the samples are taken for examination, showed a diminution of leucocytes — a hypoleucocytosis, the blood of the capillaries of the internal organs, and especially of the lungs, showed a marked increase in the number of leucocytes—a hyperleucocytosis.

There is a number of important facts which speak definitely against the essential significance which Löwit has ascribed to leucopenia. There is no reason to believe that the various substances which were shown to exercise a distinct chemotactic action on the leucocytes in the original test tube experiments should under altered conditions require the assistance of the disintegration products of the white blood corpuscles to perform this task. But apart from this it may be said that clinical experience in general speaks against Löwit's theory. In infectious diseases a hyperleucocytosis is noted very frequently, while even a temporary leucopenic stage is extremely rare.

The fact that this does not correspond with the observations made by Löwit in his experiments is readily explained when it is considered that the condition of experiment differed materially from the processes of natural disease. In the former case the experiment animal is suddenly overwhelmed by an intravenous injection of a noxious substance, which necessarily is followed by a violent acute reaction of the vascular and blood systems. In a natural infection the quantity of poison increases gradually, and only exerts its toxic influence little by little. For this reason it is probable that hypoleucocytosis is much rarer in infective processes with a normal course than under the violent conditions of an experiment.

A vast amount of literature has accumulated which deals with the clinical significance of leucocytosis, especially with regard to the infectious diseases and their various stages. To select one of the most carefully studied examples, namely, that of pneumonia, it can be said that the occurrence of leucocytosis as a constant phenomenon during the typical course of this disease is undisputed. It lasts practically right up to the onset of the crisis, and from this time onward the number of

leucocytes diminishes to below the normal level. The observations that leucocytosis may be absent in the specially severe or fatal cases are of great importance (Kikodse, Sadler, v. Jaksch, Tschistowitsch, Türk, and others).

The same observation was made in other diseases, that hyperleucocytosis is usually absent in those cases which run a particularly severe course or which are in the least atypical. It has further been shown by several observers (Löwy and Richter, M. Hahn, Jacob) that an artificial hyperleucocytosis influences the course of an infective process favourably, at all events in experiment animals.

Practical results in the treatment of disease on this basis have not been achieved, because only the specific mode of producing the leucocytes which is peculiar to each disease exercises a decided influence on the course of illness. A mere increase in the number of cells fails to effect this action.

The fact that the amount of toxin is a very important factor in determining the degree of leucocytosis is a very interesting one. As has been demonstrated by a number of experiments (Tschistowitsch, Williamson, Jacob), a small dose of toxin leads to a slight leucocytosis, higher doses produce well-marked leucocytosis, while very large quantities of toxin induce an insufficiency of the bone marrow, and consequently prevent a reactive increase of the cells in the blood. The last-named case usually gives rise directly to leucopenia. A number of analogies to these experimental observations may be found in the special pathology of the infectious diseases. The most convincing of these is included in the publications of Sonnenburg and his pupils Federmann and Kothe; these authors report that some forms of perityphlitis begin with a very high degree of leucocytosis, which may last only for a few hours, and then the numbers may fall rapidly to normal and even subnormal levels. Observations of this kind are calculated to demonstrate in a most striking manner that leucocytosis is not a purely chemotactic process, which is merely dependent on the movements of leucocytes, but that it must be a highly complicated biological

phenomenon, the form of which is largely determined by the power of reacting on the part of the bone marrow. The definition of the word chemotaxis might, it is true, be extended to include a distant action of chemical substances in general on the blood and blood-producing organs, instead of simply applying to the attraction and local movement of the leucocytes.

On the application of a suitable dose of the chemical substance a stimulation of the cells present in the bone marrow would take place, which would be evidenced by a proliferation of the marrow cells and as a rule by an increased output into the blood of these cells. When other doses are applied a hypersensibility of the medullary elements would be produced, under the influence of which the immature mononuclear forms would leave the marrow, and in this way all pronounced increase of cells in the central organs would cease. Regarded in the more extended light, the chemotaxis as defined formerly, *i.e.* the locomotion of the leucocytes, would then only form a part of the whole process.

Leucocytes may be divided from this point of view into: (1) Simple leucocytes, usually endowed with distant action (formerly spoken of as active), and (2) leucocytes without the capability of distant action (formerly termed passive). The lymphocytes would belong to the latter group.

Having regard to the fact, mentioned above, that the same substance may produce leucocytosis or not according to the dose, it can scarcely be supposed that a diminution in the number of leucocytes—leucopenia—is a process which has no connection with leucocytosis. Both conditions can very well be produced by the same cause, and must therefore be regarded as differing only in degree, in correspondence with the dose of toxin.

The close relationship may further be shown to exist in the fact that a diminution or even total disappearance of one kind of leucocyte frequently occurs even when the total number is greatly increased. In other words, there may be a leucocytosis, say, of the neutrophile cells simultaneously with a leucopenia of the eosinophiles; this occurs quite frequently. Leucocytosis

and leucopenia are thus the morphological expression of biological processes in the function of the leucocyte-forming organs. Marked diminution in number of the white blood corpuscles is very characteristic of certain diseases, especially enteric fever. The neutrophile elements are most frequently affected, especially when the disease has reached its acme and in the final stages. The conclusion to be derived from the foregoing is that the toxin of typhoid fever specially damages the function of bone marrow. This assumption has been confirmed by animal experiment (Naegeli, Studor), and has received support from the fact that a neutrophile leucocytosis does not occur in severe cases, even when certain factors are present which tend to produce leucocytosis, such as pneumonia, abscesses, turpentine injections, etc.

Considerable degrees of leucopenia are met with in severe cases of enteric fever, at the onset of morbilli, frequently in cirrhosis of the liver, as well as in severe forms of anæmia of various origin, and especially as a regular find in Biermer's pernicious anæmia, under which condition the cause of the disease does not play any part. The characteristic changes in these cases include those cells which are derived from the bone marrow,—that is, neutrophile cells, transition forms, and eosinophile cells, which are much diminished in absolute numbers, often as low as one-fourth to one-sixth of the normal number, while the lymphocytes appear in abnormally high percentages, although their absolute values correspond approximately to the normal.

A steady, slow increase in the number of the leucocytes is seen regularly during remissions in this disease.

The explanation for this peculiar character of the blood in pernicious anæmia is obvious. The leucopoiesis, like the erythropoiesis, is markedly inhibited and insufficient.

Excluding leucopenia, which would result from a destruction of a part of the white blood corpuscles (Löwit) on the ground that it is non-proven, the following causes of the phenomenon of leucocytosis may be accepted :—

1. *Abnormal distribution.*—This is rare and transitory. The leucocytes collect in the capillaries of the internal organs after intravenous injections.

2. *Abnormally small supply of leucocytes.*—

(*a*) Due to toxic, functional inhibition of the formation of leucocytes, as in infectious diseases, poisoning, and anæmia.

(*b*) Due to anatomical destruction of the leucopoietic organs, as in extensive tuberculosis or carcinosis of the lymphatic system, in which case the lymphocyte values are permanently and markedly lowered.

It has never been proved that negative chemotaxis affects the cells of the blood.

The morphological character of leucocytosis is by no means uniform, and it is therefore necessary to divide the increase of the leucocytes into various groups, according to the kind of cell which participates in the increase.

Ehrlich formerly recognised an active leucocytosis in which the cells obeyed a chemotactic law and migrated spontaneously into the blood, and a passive form in which the cells were washed mechanically into the circulation. In accordance with his view, that the lymphocytes are not endowed with any active movement, Ehrlich included all forms of lymphocytosis, including lymphatic leukæmia, among the passive leucocytoses.

The extension of the conception of chemotaxis rendered it impossible to adhere to this division, especially since the chief importance is invested in the influence exercised on the formation of cells in the organs, or in other words in organ function.

Whenever any kind of cell is present in the blood in increased numbers a more intense activity of the organ in which these cells are formed undoubtedly takes place.

Leucocytosis may be divided according to the class of cell which is increased. An increase of more than one kind of leucocyte may not infrequently be met with in one disease.

A.—Polymorpho-nuclear Neutrophile Leucocytosis

By far the most common form of leucocytosis is that in which the polynuclear neutrophile leucocytes are increased in numbers. A large number of the most different conditions and influences lead to its occurrence.

Virchow, the discoverer of leucocytosis, was of opinion that leucocytosis depended on an increased stimulation of the lymphatic glands. He taught that the stimulation of the lymphatic glands consists in "the taking on of an increased production of cells and in the enlargement of the follicles, in which, after a time, many more cells are contained than before." The swelling of the lymphatic glands was supposed to induce an increase in the number of lymph corpuscles, and from this followed an increase in the number of white blood corpuscles in the blood.

Ehrlich's researches necessitated the relinquishing of this view, since they showed that the migration of the polynuclear neutrophile cells was to a large extent responsible for the leucocytosis. Exact cell counts were first carried out by Einhorn under Ehrlich's direction, and later on the results obtained were generally confirmed. In correspondence to the increase which was limited to the neutrophile corpuscles, the percentage of the lymphocytes was always found to be diminished, at times to such an extent that these cells only represented 2 per cent. or less of the total number of white cells. It must, however, be remembered that the percentage of the lymph cells may be markedly diminished without their absolute number being altered. But it has frequently been found that, associated with the polynuclear leucocytosis, a decrease in the absolute number of lymphocytes takes place.

The transition forms often show considerable increase in neutrophile leucocytosis, and single neutrophile myelocytes may be found among these cells. This increase may even reach a moderately high percentage. Apart from the appearance of myelocytes and of numerous immature leucocytes, the fact that a few nucleated red blood corpuscles may be found in the peripheral

blood in the absence of anæmia, speaks strongly in favour of the view that the bone marrow is working at very high pressure.

In the ordinary forms of polynuclear neutrophile leucocytosis the eosinophile cells are usually absolutely diminished in number, as Ehrlich pointed out in his first publication on this subject. The diminution is frequently a considerable one, and at times these cells may be absent altogether.

In a few pathological conditions there may, however, be an increase of eosinophile cells in association with a polynuclear neutrophile leucocytosis. This will be dealt with under a separate heading.

Polynuclear neutrophile leucocytosis — leucocytosis *par excellence*—may be divided into several groups according to its clinical occurrence. The following forms are recognised:—

(i) PHYSIOLOGICAL LEUCOCYTOSIS

The leucocytosis of digestion must be included in this group. This is said to occur after the ingestion of albuminous food. Japha, however, is of opinion that this is merely a physiological diurnal variation. While the older authors found that the lymphocytes were increased, more recently an increase of the neutrophiles has been said to occur.

According to the more recent investigations, the leucocytosis of pregnancy only affects primiparæ regularly, and even in them is but slight. It affects the neutrophiles chiefly.

The leucocytosis of new-born infants is only marked in the first four days of life and is of a neutrophile character.

Increased numbers of leucocytes can also be found in the peripheral blood after bodily over-exertion and thermic stimuli. It is, however, possible that vasomotor changes may be responsible for this, at all events in part.

(ii) PATHOLOGICAL LEUCOCYTOSIS

1. The increase in the number of the polynuclear cells which occurs in infectious processes has been called inflammatory, in accordance with the principle: *a potiori fit denominatio.* They

are nevertheless inflammatory toxic processes, since the toxins of the infective microbes determine the character of the leucocytosis. This has been proved beyond all doubt by innumerable experimental researches. It is particularly important to note that the majority of febrile conditions, *e.g.* pneumonia, erysipelas, diphtheria, septic conditions of various origin, acute articular rheumatism, etc., are accompanied by a definite more or less marked leucocytosis. Uncomplicated enteric fever and morbilli alone occupy an exceptional position in this connection. The absolute number of white blood corpuscles in these diseases is decreased often at the cost of the polynuclear neutrophile cells. The reader is referred to the various text-books on hæmatology, and to the publications of Türk, Stienon, Schindler, Reckzeh, Zappert, and others for the details with regard to this behaviour, and also for the course and termination of leucocytosis associated with the infectious diseases.

The chronic infective processes, and above all tuberculosis, produce extremely slight changes in the blood, so that no constant variations from the normal can be ascertained.

As a rule the acute infectious diseases begin with a considerable neutrophile leucocytosis. This may, as is the case in measles, fall during the incubation stage, or, as is the case in typhoid fever, may last for an extremely short time; as a rule it lasts for a considerable time. During this stage the lymphocytes are diminished and the eosinophiles are either greatly reduced in number or disappear from the blood altogether. As the infection passes off the lymphocyte curve rises again, as does that of the eosinophiles, and during convalescence the curves may reach a level higher than normal, which is spoken of as post-infective lymphocytosis and eosinophilia. This is in accordance with a general biological law which states that a diminished activity of a tissue is followed by an activity after recovery which exceeds the normal.

2. Toxic leucocytosis is met with especially in poisoning with the so-called blood poisons. The majority of blood poisons, such as potassium chlorate, the derivatives of phenyl-hydrazin,

pyrodin, phenacetin, etc., in general appear to produce a considerable increase of leucocytes in human beings, as well as to destroy the red blood cells. This has been confirmed experimentally. It must further be mentioned that marked leucocytosis may be produced by the injection of tissue extracts containing nuclein and of nuclein alone.

3. The leucocytosis of anæmic conditions and hæmorrhages is especially well known as post-hæmorrhagic leucocytosis. It indicates a strong bone marrow reaction, which affects *inter alia* the white blood corpuscles.

4. The leucocytosis of malignant tumours is not constant, but may be very marked. The cause must be sought in various factors, *e.g.* in the absorption of toxic substances in the decomposition of fouling discharges.

A particularly great increase is met with in metastases occurring in the bone marrow. In these cases nucleated red blood corpuscles and numerous myelocytes may pass into the blood, so that the blood presents an appearance similar to that seen in leukæmia.

The so-called cachectic or agonal leucocytosis does not depend on cachexia or agony as such, as used to be held. It is frequently absent in both conditions. When it is present it is the result of the condition producing the cachexia or agony.

It is quite clear that the conditions of the cells of the blood in the various diseases may be of considerable clinical importance. It is only possible to touch on a few of the more salient points in this place, and to refer the reader to the text-books on morphological hæmatology for further details.

(*a*) The great importance in the differential diagnosis which attaches to the leucopenic blood condition in enteric fever as contrasted with nearly all other infectious diseases.

The early diagnosis of measles during the incubation period.

The recognition of trichinosis and the extraordinarily easy differential diagnosis between trichinosis and typhoid fever, which used to be difficult to make.

The importance of leucocytosis for the recognition of suppurative processes, and of the tendency of these processes to become extended.

(*b*) The prognostic importance of changes of this kind in the blood, *e.g.* the absence of leucocytosis in severe diseases, in which a marked increase of the neutrophile cells would otherwise have been expected from the nature of the process, would indicate an insufficiency of the bone marrow, and would therefore point to a very severe affection (examples: pneumonia, perityphlitis, peritonitis, etc.).

Ehrlich teaches that the origin of polymorpho-nuclear neutrophile leucocytosis lies in the bone marrow. It is not necessary now to support this view with as many arguments as it was ten years ago. This does not mean that there are no persons left who believe that the origin of leucocytosis should be sought for in the inflammatory and suppurative foci, in the intestinal wall, in the mucous membrane of the uterus, and so on, but views of this kind may be treated to-day as curiosities. The origin of the neutrophile elements in the bone marrow is firmly established, because in no other organ are the precursors of the neutrophiles of the blood—the myelocytes—to be found. They are present in these organs in tissue formations in very large numbers. In this situation all forms of transposition of the nucleus, and all forms intermediate to those of the cells found in the blood, are present. Mitosis is found in this situation, and even if there is no doubt that in certain pathological conditions myeloid formations appear in other organs, the functional significance of these extraneous formations is only very subordinate, save perhaps in leukæmia.

B.—Polynuclear Eosinophile Leucocytosis.

After Ehrlich had demonstrated the constant increase of the eosinophile cells in leukæmia, a long time passed before eosinophilia was found in any other form of disease, the characters of which differed essentially from leukæmia. The first advance in this direction was made by Friedrich Müller, on whose advice

Gollasch examined the blood of asthmatics and found therein a distinct increase in the number of eosinophile cells. Following this, H. F. Müller and Rieder discovered that eosinophilia exists with great frequency in children and in connection with chronic splenic tumours. Next followed the well-known work of Edm. Neusser, in which he proved that a very marked increase of the oxyphilic elements occurs in pemphigus and almost simultaneously analogous observations by Canon in chronic skin diseases. It is only necessary to mention the comprehensive survey of this subject published by Zappert and K. Meyer from among the enormous number of other communications.

By the term eosinophilia is meant an increase of the cells of the blood affecting the eosinophile polynuclear cells alone. It is quite impossible to confuse this form of leucocytosis with leukæmia, because a whole series of other characteristic signs is necessary for the recognition of the latter. These signs will be dealt with in the following chapter. It is not permissible to regard the presence of mononuclear eosinophile cells in the blood as absolute proof of a leukæmia, as some authors have done, since these cells are found in some cases of ordinary leucocytosis.

The increase in the number of the eosinophile cells is in every case not only a relative, but also an absolute one. The percentage of these cells under normal conditions is from 2 to 4 per cent., but rises in eosinophilia to 10, 20, 30 per cent. and higher.

Polynuclear eosinophile leucocytosis is found in manifold pathological conditions, as well as in healthy children, and for the sake of clearness these conditions may be divided into the following groups.

1. **Bronchial Asthma.**—In this disease an increase, which is frequently very considerable, in the number of eosinophile cells in the blood was first discovered by Gollasch, and this was confirmed later by a large number of other observers. The percentage may rise to 10 and 20 per cent. or higher. In hay asthma and hay fever absolutely similar conditions are met with. (For the special clinical course of eosinophilia in asthma, see below.)

2. **Pemphigus.**—Neusser was the first to find an extraordinarily marked almost specific eosinophilia in some cases of pemphigus. This interesting observation has been confirmed by a number of workers, among whom Zappert should be mentioned. The latter found in one case as many as 4800 oxyphile cells in a c.mm. of blood.

3. **Acute and Chronic Skin Diseases.**—Canon was the first to notice that in a large number of skin diseases, especially in prurigo and psoriasis, the eosinophile cells may be increased up to 17 per cent. Canon pointed out one remarkable fact, that it is not so much the kind of disease or its local intensity as the extent of the process which determines the degree of the increase of the eosinophile elements. In one case of acute very extensive urticaria A. Lazarus found the eosinophiles representing 60 per cent. of all the leucocytes; within a few days this enormous number of eosinophile cells diminished to the normal level.

4. **Helminthiasis.**—The first observations with regard to the occurrence of eosinophilia in helminthiasis emanated from H. F. Müller and Rieder, who demonstrated fairly high values (8·2 and 9·7 per cent.) in two men suffering from ankylostomum duodenale. Shortly afterwards Zappert reported that he had found a considerable increase of these cells in the blood of two further cases of the same disease; the value reached 17 per cent. He also found Charcot's crystals in the fæces. In a third case of ankylostomum, however, Zappert failed to find the eosinophiles of the blood increased, or any crystals in the fæces. Seige also found similar conditions.

Leichtenstern, whose work on parasitology is well known, has published an extensive essay on this important subject. Under his direction Bücklers discovered the interesting fact that ankylostomum does not take an exceptional position among the diseases produced by worms with regard to the production of eosinophilia. He found that all the forms of worms observed in the Cologne Hospital, from the thread-worm which is generally regarded as harmless to the pernicious strongylus, produced an increase of the eosinophile cells in the blood, which at times

reached a great height. Bücklers reported that he found 16 per cent. of eosinophiles in oxyuris, 19 per cent. in *Ascaris lumbricoides*, and Leichtenstern announced in a later communication that he had come across a case of ankylostomiasis with 72 per cent. of eosinophiles and one case of *Tænia mediocanellata* with 34 per cent.

It is very remarkable that Leichtenstern was able to find large numbers of eosinophile cells especially in those cases in which the fæces contained quantities of Charcot's crystals. Since eosinophile cells and Charcot's crystals have on other occasions been frequently found associated with one another (*e.g.* in bronchial asthma, nasal polypi, in myelæmic blood and bone marrow) it is quite reasonable to accept Leichtenstern's thesis, that eosinophile cells may be present in the intestinal mucosa in ankylostomiasis. This has, however, not yet been demonstrated.

The almost constant increase in numbers of eosinophile cells in trichinosis has proved to be of great diagnostic value. This fact was first discovered by T. R. Brown, working under the direction of Thayer. He found the eosinophile value as high as 68 per cent. and the absolute value as high as 20,400.

Since Brown's communication, analogous observations have been made by Schleip among others in a large epidemic, and by Opie and Stäubli in experimental trichinosis. It has on many occasions been possible to clear up clinically obscure or doubtful cases by means of hæmatological examination.

5. **The Post-infective Form of Eosinophilia** (after the termination of various infectious diseases).—As has been mentioned in the chapter dealing with polynuclear neutrophile leucocytosis, a relative diminution of the number of eosinophiles or even a total disappearance of these cells may be seen at the height of the fever in the infectious diseases, with the one exception of scarlatina. In the post-febrile stages, however, the highest values which can still be considered normal are often met with, or there may even be a distinct eosinophilic leucocytosis. When this occurs it is usually quite moderate in degree.

Not infrequently, however, very considerable increases may

be noted, as in the case of typhoid fever, when the numbers may be as high as from 1200 to 1500; this can be seen more often if the blood is examined for a considerable time after recovery (two or three months).

The eosinophilia appearing after injections of tuberculin should also be included in this group. It must, however, be mentioned that, according to Fauconnet's researches, this condition cannot be regarded as proved.

6. **Malignant Disease.** — A number of authors have observed a moderate degree of eosinophilia in the cachexia of malignant disease; the values do not exceed 7 to 10 per cent. in these cases. Reinbach only found the eosinophile cells increased four times in forty cases of this kind. The values found were 7·8 per cent. in sarcoma of the forearm, 8·4 per cent. in sarcoma of the leg, and 11·6 per cent. in an abdominal malignant tumour. He also recorded a case of lymphosarcoma of the neck with secondary growths in the lymphatic glands, in which an enormous increase of the white blood corpuscles and especially of the eosinophile cells was present. The absolute number of the latter at one time was 60,000, which is equivalent to three hundred times the normal value; such an increase has never been seen in any condition save perhaps leukæmia.

A moderate degree of increase is not uncommon in the early stages, especially of carcinoma. The more the cachexia advances the greater is the tendency for the values to sink below the normal level.

In the immediate neighbourhood of cancerous nodules veritable nests and collections of eosinophile cells are often met with.

7. **Compensatory Eosinophilia** (after elimination of the spleen).—This form of eosinophilia has been dealt with in detail in the chapter on the function of the spleen. It has been pointed out that the increase of eosinophile cells which has been found by Rieder, Weiss, and others in chronic splenic tumours may be attributed to the elimination of the spleen. The data with regard to this point need supplementing.

8. **Medicamentous Eosinophilia.**—The only observation of this kind published hitherto was made by von Noorden. He found eosinophilia up to 9 per cent. in the blood of two chlorotic girls after they had taken camphor. The phenomenon could not be induced in other patients. An increase of the eosinophiles can be noted in connection with other preparations; in these cases there is always a preliminary decrease. As is the case in the post-infective form of eosinophilia, this condition is undoubtedly due to a toxic (toxic-infective) influence on the production of the eosinophile cells in the bone marrow, and not to a chemotactic effect. The function and cell production of the marrow is first diminished, and after recovery takes place the function is likewise restored and may even be increased above the normal level. It is possible that a direct casting out of eosinophile cells occurs without any destruction of the same in the early stages, or in other words that there is a negative chemotaxis in this case; but this question requires further careful investigation.

9. **Nervous Eosinophilia.**—The origin of this phenomenon is still obscure, but there is no doubt that it takes place. Quite considerable increases in number of eosinophile cells are seen not infrequently in neurasthenia. The author has found as high a value as 10 per cent. in nervous diarrhœa associated with colic.

The analogy with the conditions obtaining in bronchial asthma is so obvious that it is unnecessary to dwell upon it.

10. **Scarlatinal Eosinophilia.** — Scarlatina is the only bacterial infection which yields an increase of the eosinophiles at the height of the fever. This usually takes place on the second day of the fever. As a rule, only moderately high numbers are found, such as 500 to 1000, but at times they may be as high as 2000 or 3000 during the acute stages. It is quite possible that this form of eosinophilia is related in some way to the exanthem; in scarlatina without a rash no increase is observed.

11. **Leukæmic Eosinophilia.**—The increase in the numbers of the eosinophile cells in myeloid leukæmia is practically a

regular occurrence, and the absolute numbers at all events are often very high. This subject will be considered in detail subsequently.

Various theories have been enunciated with regard to the origin of the polynuclear eosinophile leucocytes. Ehrlich found it necessary to establish his view of the bone marrow genesis of these cells in the first edition of this work by the application of much ingenuity. It is no longer necessary to defend this view. The theories which assume that the eosinophile cells are derived from the neutrophile cells within the blood vessels are not supported by any histological evidence. Such an occurrence can never have been observed, and this view need therefore not be taken seriously.

The bone marrow genesis is quite clear, and may be demonstrated histologically by means of modern section staining. As is the case with the neutrophile cells, the bone marrow is the only site where eosinophile myelocytes occur under normal conditions and in which all the forms intermediate between the myelocytes and the polymorpho-nuclear cells are met with.

Several authors believe that the eosinophile cells can be formed locally. This is, however, not the case under ordinary conditions. The infiltrations around the trichinæ, the enormous peribronchial collections in asthma, and the collections in the intestinal walls have been proved to be chemotactic, since only polymorpho-nuclear cells and no myelocytes are present. Mononuclear eosinophiles are, it is true, seen in sputum, but these cells are involution forms, for they are not so large as myelocytes. The nucleus is very small, and it is not possible to demonstrate a chromatin network in them.

Nevertheless, under quite exceptional conditions, an extra-medullary genesis of the eosinophile cells may take place, but not from any chance connective-tissue cell. This can only occur in connection with a vessel, and the formation takes place either from adventitial cells or in consonance with Schridde's views, from "cells of the vascular wall." Such formations, however, are never exclusively eosinophile. They always reveal myeloid com-

plexes, in which neutrophile cells and even nucleated red cells are also formed.

These extramedullary myeloid foci are very widely disseminated and frequent in the embryo. In some animals they are also met with in the adult. They are found in man in infective processes, in intoxications, and in anæmias, more especially in leukæmia, and may be very extensive.

That the collections in bronchial asthma are really chemotactic is proved by the way in which the proportional numbers of the eosinophile cells vary to a large extent. According to Heineke and Deutschmann, the number of eosinophile cells in the blood in bronchial asthma sinks very materially after the attack. This would imply that a temporary functional eosinophilia is present which, as is the case in the analogous conditions of the neutrophiles, can only be the result of an increased activity of the bone marrow.

The question which cells produce chemotactically active substances out of their disintegration products is a very important one, but is one which cannot be decided at present with certainty. The ordinary pus cells and the lymphocytes do not appear to produce any such substances on disintegration ; on the other hand, there are many reasons for believing that the dissociation products of epithelial cells and of epithelioid cells act chemotactically. In this way the frequent occurrence of eosinophilia in the various forms of skin diseases might be explained. The same explanation would hold good for the appearance of local collections of eosinophile cells in all atrophic conditions of the gastric, intestinal, and bronchial mucosa, and also for the increase of these cells in the neighbourhood of carcinomata. A further argument in favour of this view is the fact that the eosinophile cells are more numerous in bronchitis and asthma when the secretion contains but few pus cells. In the last place, the observation made in scarlatina, that when no rash is present no eosinophilia takes place, also speaks in favour of this view.

On the other hand, there is no doubt that foreign substances circulate in the body which are able to exercise a positive chemotactic influence on the eosinophile cells. Goldmann found in

sections of the pancreas which contained *Proteus sanguineus*, that the eosinophile cells were markedly increased in number in the neighbourhood of the encapsuled parasites, while he sought for these cells in vain in other areas. Opie came across similar conditions in connection with encapsuled trichinæ, but Sträubli emphasised the presence of interstitial foci of eosinophile cells under these conditions.

Pröscher reported a very striking condition, by producing an eosinophilic pleurisy experimentally by means of extracts of the tape-worm.

In this connection the observations made in the various forms of helminthiasis (see p. 166) are of considerable importance. It was formerly thought that the action of worms was a purely local one. The view that their action is due to toxic substances which they produce is now gaining considerable support. Linstow has pointed out that the general typhoid condition, and also the fatty degeneration of the liver and kidneys, *i.e.* of organs in which the *Trichinæ* are not found, necessitate the assumption of a toxin in trichinosis.

The symptoms produced by the *Bothriocephalus latus* are now regarded as being due to a specially produced poison. Even the ordinary tape-worms effect a damage to the organism not infrequently, and this damage is to be ascribed to the production of a poison (Peiper).

These considerations justify the deduction that tape-worms not only take up substances from their hosts, but also give off other substances which may be taken up by the intestine of the host and which may exercise a distant action. One sign of this distant action, as Leichtenstern has pointed out, is the appearance of eosinophilia of the blood. The author believes that the above-mentioned facts absolutely exclude the possibility that the substances which attract the eosinophile cells are identical with the substances which produce anæmia. Several observations, such as that of the absence of eosinophilia in *Bothriocephalus anæmia* (Schauman and Naegeli) point to the probability of the existence of two separate functions. At all events, the substance which

produces the eosinophilia is much more widely distributed than the substance which is responsible for the anæmia.

In order to consider how polynuclear eosinophilia is produced, it may be advisable to have regard, in the first place, to an experiment which was performed by E. Neusser. Neusser found that the contents of the bullæ in a case of pemphigus consisted almost exclusively of eosinophile cells. The blood of this patient showed a considerable increase of eosinophiles. Neusser thereupon produced a non-specific inflammatory blister by means of a vesicant, and found that the cellular contents of this blister were exclusively polynuclear neutrophile pus cells such as are met with in all simple inflammations.

Leredde and Perrin met with conditions which were absolutely analogous to those of Neusser's case, without the assistance of any experimentally produced lesions, in what is known as Dühring's disease. The vesicles which occur in this dermatosis contained only polynuclear eosinophile cells as long as the fluid remained clear. In a later stage, as is usually the case, bacteria invaded the vesicles, and when this occurred they were found to contain cells with neutrophile granules only.

Neusser's experiment and Leredde and Perrin's observations can only be explained, in accordance with the modern views of the nature of suppuration, by assuming that the eosinophile and the neutrophile cells possess chemotactic susceptibility. This view has already been supported in this work. According to this conception, the eosinophile cells would only migrate towards those sites which contain substances which specifically stimulate these cells. All the experiments and clinical observations on eosinophilia which have hitherto been recorded may be explained *lege artis* on this assumption. Neusser's experiment may be analysed as follows. A substance is present in the bullæ of the pemphigus case which attracts the eosinophile cells chemotactically. The eosinophile cells which are normally present in the blood migrate from the circulation and produce an eosinophilic suppuration. When the disease is not very severe the process may be regarded as being limited to a great extent to the localised phenomenon.

A totally different picture is developed, however, when the disease involves considerable areas of the body. Under these conditions a large quantity of the specific active agent is taken up into the blood stream by diffusion and absorption, and from this situation it exerts a strong chemotactic action on the physiological depots of the eosinophile cells, *i.e.* on the bone marrow. This leads to a more or less marked increase of the eosinophile cells in the blood. The bone marrow, in consequence of the increased migration, is stimulated to produce more cells, in accordance with general biological laws, and thus retains the power, even when the disease lasts for a long time, of keeping up a continuous eosinophilia.

Other clinical experiences may also be explained satisfactorily in this manner. Gollasch found that the sputum of asthmatics only contains eosinophile cells in addition to Charcot's crystals. It must therefore be supposed that the interior of the bronchial tree contains a substance which attracts the eosinophile cells. The close relationship which has been shown by numerous clinical observations to exist between the severity of the disease and number of eosinophile cells in the blood may also be regarded as pointing to this view. von Noorden was able to show that the eosinophile cells in the blood are more numerous about the time of an attack than when a considerable time since the last attack has elapsed. They were present in especially large numbers when the attacks followed one another rapidly during the course of several days. That the increase in number of the eosinophile cells in this case depends directly on the attack and is not merely a sign of a persistent anomaly of constitution is proved by a case which von Noorden reports. During the attack he found 25 per cent. eosinophiles, while a few days later he only found one single cell in twelve cover-slip specimens, which means that there was actually a diminution of this group of blood cell.

Canon had the same experience in skin diseases. He was able to show that the degree of the eosinophilia depended on the local extent of the affection rather than on the intensity.

This means that the eosinophilia depends on that factor which determines the quantity of the specific agent in the blood.

All those laws which have been described as governing neutrophile leucocytosis are applicable in the case of eosinophile leucocytosis also. One of the most important facts in this connection is that no eosinophilia occurs when the bone marrow function is paralysed, even if chemotactically active substances are present. Sträubli produced very severe experimental trichinosis which ran its course to a fatal termination with a complete absence of acidophile cells; while Liermberger's ankylostomum patients only showed low percentages of eosinophile cells during a very severe illness. On the application of arsenic the number increased from 3·2 per cent. to 33·7 per cent., in spite of the fact that the worms were not driven out. Leichtenstern noticed on a previous occasion that a croupous pneumonia in an ankylostomum patient could depress the number of eosinophile cells from 72 per cent. to 6 or 7 per cent., and that the former values were closely approached at a later date again.

The presence of chemotactically active substances is therefore not to be regarded as the final determining cause. A capability of reacting on the part of the bone marrow in addition is necessary. The quantity of toxin may be even too large, as in experimental trichinosis, so that the eosinophilia may be partly or completely prevented. Opie was able to show that an overdose of the agent does not produce a stimulation of the marrow, but actually kills the cells.

These considerations prove that the eosinophile curve records the function of an organ, and it therefore follows that it is quite impossible for the changes in the blood and tissues to depend on a local histogenic formation.

C.—Mast Cell Leucocytosis

The increase of this form of white blood corpuscle is undoubtedly rare, and with the exception of the case of

leukæmia this increase is small. It must, however, be borne in mind that normal blood contains a very small number of these cells, so that even a triple or quadruple increase would scarcely be of importance in consideration of the number of the other cells.

Mast cell leucocytosis has been seen in the following conditions apart from leukæmia. In skin diseases, in suffusion of milk of the human breast (Unger), in *urticaria pigmentosa* (Sabrazes). Levaditi has produced mast cell leucocytosis in animals.

The real mast cells of the blood with their characteristic polymorphous form of nucleus must not be confused with the mononuclear mast cells of the tissues, which are often found in large numbers in chronic inflammations, in indurations of the lung, in skin diseases, and in inflamed lymphatic glands. These latter cells are absolutely different structures, and possess practically no relationship to the mast cells of the blood. The only character which they possess in common with the blood cells is the presence of basophile metachromic granulation. The tissue cells have small round nuclei, which take on the stain of nuclear dyes well.

The mast myelocytes of the bone marrow, which represent the precursors of the blood mast cells, are quite different from these tissue mast cells, and it needs only slight histological knowledge to see that an intimate connection between these two kinds of mast cells cannot exist.

VI.—LEUKÆMIA, OR LEUCOCYTHÆMIA

The interest which the investigator and the clinician has taken in leukæmia has not diminished during the past ten years. This is shown by the innumerable publications on the subject, and by the many theories which have been evolved on the pathogenesis of this remarkable disease.

Much that has been suggested in this connection has enjoyed but a short life and has soon been forgotten. It has been shown

that the study of the blood and its cells is incapable of throwing light on the genesis of the disease, but that advance can only be expected from a most careful examination of the organs, in combination no doubt with a minute analysis of cells in the microscopical sections of these organs. The results of this kind of investigation became so plentiful when this was recognised that our knowledge of leukæmia thereby has been very materially extended.

It may be worth while first to follow the march of research since the discovery of leukæmia. In this way those problems which engage the attention of the investigator at present will present themselves automatically.

At first a lymphatic, a splenic, a spleno-medullary, and a pure medullary or myelogenous form of leukæmia or leucocythæmia were distinguished on the basis of their clinical appearances. This classification depended on purely external and gross signs which could not be recognised in hæmatology.

Such a classification takes the extent of the changes in the organs into consideration in the first place, but not the nature of these changes. It ignores the most important factor of all, the kind of cell proliferation.

Neumann was the first to show that in lymphatic leukæmia the lymphatic proliferation is not limited to the lymphatic glands, but may affect the spleen and bone marrow. These proliferation processes may cause an enormous enlargement of the spleen, without any change taking place in the specific character of the leukæmic process or of the appearances of the blood. In spite of the splenic tumour, the case is one of lymphatic leukæmia. In ordinary clinical terminology such a case is spoken of as "lymphatic splenic leucocythæmia." The unreliability and incorrectness of such a term can best be demonstrated in another form of leukæmic proliferation. The liver may become enlarged to the size of a large tumour in lymphatic leukæmia by the production of lymphomata, and logically speaking this should be termed a "lymphatic hepatic leukæmia." This term would not be so prone to lead to error as the term

"lymphatic splenic leukæmia"; for no one would imagine that in the former the liver cells pass over into the circulating blood, while the latter term suggests that the specific splenic cells take a part in the changes in the blood.

The recognition of a pure splenic form of leukæmia is to be regarded as unjustifiable from the point of view of hæmatological and histological investigations. After what has been said with regard to the physiological participation on the part of the spleen in the formation of blood, the probability of a blood change which is specifically due to an affection of the spleen is almost excluded. This view has received full confirmation from the results of pathological investigations.

There is not a single case in the whole of the literature of the subject in which a pure splenic leukæmia could be accepted.

The conditions obtaining with regard to myeloid leukæmia are similar to those mentioned above, in so far as the occurrence of myeloid tissue in the spleen and lymphatic glands is concerned. Since the proliferation of this tissue and not the accompanying swelling of the spleen or lymphatic glands is the specific factor in the process, it follows that the term "spleno-medullary" or "medullary lymphatic" leukæmia is also illogical and likely to lead to error.

Ehrlich therefore recognised from the hæmatological standpoint two forms of leukæmia.

1. Leukæmic processes with proliferation of lymphatic tissue —**Lymphatic Leukæmia.**

2. Leukæmic processes with proliferation of myeloid tissue— **Myeloid Leukæmia.**

If it be found advisable, there would be no objection to indicate the accompanying clinical signs by the addition of words which would not give rise to misunderstanding; for example, "lymphatic leukæmia with swelling of the spleen or of the liver," or "myeloid leukæmia with enlargement of the lymphatic glands," and so on.

This classification, which was made on the basis of

cytological considerations, has proved to be absolutely satisfactory in the light of subsequent histological researches. The study of the organs showed quite definitely that there are only two forms of leukæmic proliferation. In the one form the lymphatic tissue and on the other the medullary tissue is affected in correspondence with the fact that there are only two forms of tissue which form leucocytes normally. Before these aspects of this disease are dealt with it may be advisable briefly to survey its clinical manifestations, since, in view of the radical differences of the clinical appearances of the two forms of leukæmia, both should be recognised.

Lymphatic leukæmia may be divided into two clinically distinct forms. First, acute lymphatic leukæmia may be recognised by its rapid course, by the small degree of swelling of the spleen, by the tendency of petechial hæmorrhages to appear, and by the general hæmorrhagic diathesis. This form of disease has given the majority of clinicians the impression of an acute infective process on account of its fulminating course.

The second form of lymphatic leukæmia is distinguished from the preceding form by its chronic, frequently protracted course. The spleen usually participates in the disease, in taking on a very considerable swelling. Hæmatologically, all forms of lymphatic leukæmia are characterised by a marked predominance of lymph cells. Either the small or the large lymphocytes may be present, or a variable mixture of both forms. It must be emphatically pointed out that the preponderance of large lymph cells is by no means characteristic of the acute form of lymphatic leukæmia, since the same blood changes are found in very slowly advancing chronic cases. In a case of this kind, which was being treated in Gerhardt's clinic, all the observers who examined the blood (Grawitz, von Noorden, Ehrlich) were able to find the large cells during the whole course of illness.

The histological examination of the organs has yielded an indisputable explanation of the origin of this form of leukæmia. The foci of lymphatic production are not only very extensive,

but, as is proved by the occurrence of mitosis, are in a state of considerable activity. The disease therefore depends on an enormous increase in the output of cells, and the cells thus formed are passed over to the blood obviously in the same way as under normal conditions.

Chemotactic laws do not come into play at all, neither are the cells passively washed out of the organs. Hyperfunction of the tissue is the characterising factor of the process.

Myeloid leukæmia presents a picture which is totally different from every point of view.

In former years great difficulty was experienced in differentiating between myeloid leukæmia and simple leucocytosis; the two phenomena were even regarded as different degrees of the same pathological process, and it was thought that when the proportion of the white to the red blood corpuscles exceeded a definite limit (1 : 50) leucocytosis ended and leukæmia began. The fundamental differences of the two conditions were only recognised when the cells were analysed with the assistance of staining methods. Leucocytosis is now recognised as a condition in which the normal polynuclear neutrophile leucocytes are merely increased in number, while in myeloid leukæmia elements are introduced into the blood stream in large numbers which are not normally present in it. The cell forms which are thus introduced into the blood are so characteristic that the diagnosis of leukæmia is possible even in those very rare cases in which the total number of white blood corpuscles is not materially increased or is actually diminished.

It is, however, necessary in cases of this kind to exercise a critical judgment and to rely on experience, since marked disturbances of leucopoiesis may be present in severe anæmias without the condition necessarily being leukæmic.

It is possible with the assistance of modern hæmatological technique, in accordance with Ehrlich's principles, to recognise leukæmia with certainty in practically every case from the appearance of the blood. Difficulties are only temporarily met

with in those cases in which either the disease is still in a very early stage—this stage, however, is very rarely seen—or when the leukæmic characters are temporarily obscured by the occurrence of complications such as an infective disease.

The opposition to the recognition of the changes in the blood in this disease has now been finally removed. Although this found its way into the text-book on hæmatology of ten years ago (v. Limbeck), the arguments used then seem quite incomprehensible now.

The microscopical appearances of myeloid leukæmia are characterised by an increase in the number of white blood corpuscles, which is almost always considerable, and by the variegated and changeable character of the cells. The latter is due to the complication of several anomalies, which consist in :—

1. That besides the polynuclear cells their precursors, the mononuclear granulated leucocytes, the myelocytes circulate in the blood ;

2. That in the increase of the white blood corpuscles all three types of granules are met with, *i.e.* the neutrophile, the eosinophile, and the mast-cell granules ;

3. That atypical forms of cells, *e.g.* dwarf forms of various kinds of white blood corpuscles and also mitotic figures are seen ; and

4. That the blood always contains nucleated red blood corpuscles, often in great numbers.

1. It is advisable to begin by dealing with the **Mononuclear neutrophile cells, Ehrlich's Myelocytes.** These cells are present in such large numbers in the blood of medullary leukæmia that they give the whole picture, at all events in the later stages, a predominating mononuclear character. Under normal conditions the myelocytes only occur in the bone marrow, as has been stated repeatedly, and never in the circulating blood. The signal diagnostic importance of the presence of these cells is not detracted from by the fact that they occur temporarily in some other conditions. Even if they are found as one of the signs of a general leucocytosis in the critical period of a pneumonia, it is unlikely that the condition could

be confused with the blood changes of leukæmia. This is safeguarded: (1) By the much smaller increase of the white cells generally; (2) by the diminution of the eosinophile and mast cells; (3) by the predominating polynuclear character of the leucocytosis, which is not obscured by the presence of a small number of myelocytes; and (4) by an incomparably smaller absolute number of myelocytes. If Türk's most extreme case be taken, namely, one of croupous pneumonia in which the percentage of the myelocytes reached as high as 11·9 per cent. of the total number of leucocytes, it will be seen that the absolute number of these cells per cubic millimetre was 1000 at most. This number is one which cannot compare with the number of myelocytes in leukæmic blood, which may reach in an average and certainly not exceptional case, 50,000 to 100,000 per cubic millimetre and higher.

Some difficulty may be experienced in connection with the so-called atypical leukæmias. The conditions obtaining with regard to this form are so complicated that it appears inadvisable to discuss them in detail in this work. The author therefore prefers to reserve this for a further communication on the subject. He also refers the reader to the chapter dealing with this subject in his text-book (page 364).

2. **The Mononuclear Eosinophile Cells.**—Mosler described large coarsely granulated cells, medullary cells, as characteristic of the myelogenous form of leukæmia even before the introduction of modern staining technique. These cells must be regarded as being to a great extent identical with mononuclear eosinophile cells, to which Müller and Rieder called attention as a special form of cell, and which they appropriately described as the eosinophile analogies of the myelocytes. These cells are large, rather bulky elements with oval nuclei which stain feebly. In spite of the fact that these cells are undoubtedly a valuable sign of a leukæmic affection, their importance in the diagnosis of this condition is not so great as that of the mononuclear neutrophile cells, on account of the numerical superiority of the latter. It is not permissible to diagnose a

leukæmia alone from the presence of "eosinophile myelocytes," because they do occur, albeit in small numbers, in other affections.

3. **The Absolute Increase of the Eosinophile Cells.**—Ehrlich has always taught, since he first published his opinions on leukæmia, that the absolute number of the polynuclear eosinophiles is always much increased in myeloid leukæmia. This statement of Ehrlich's did not remain uncontradicted. von Limbeck thus speaks of the "alleged" increase of the eosinophile cells in his text-book. It was chiefly the well-known work of Müller and Rieder which stimulated this opposition and which awakened doubt with regard to the diagnostic significance of the eosinophile cells. These authors, however, founded their opposition on false premises.

Ehrlich did not speak of an increase in the percentage of eosinophile cells, but only of an increase in their absolute numbers. Even if a normal percentage of eosinophiles is found in a case of leukæmia, this must indicate a great increase in the absolute numbers, and Müller and Rieder would have been able to have confirmed Ehrlich's statements if they had only calculated the absolute numbers in their cases. Out of the seven cases given in the work bearing on this subject, only three are given in sufficient detail to enable the absolute number of the eosinophile cells to be calculated. From these data the values are:—

Case 29.—3·5 per cent.	eosinophiles	= 14,000	per c.mm.
Case 30.—3·9 per cent.	,,	= 8000	,,
Case 31.—3·4 per cent.	,,	= 11,000	,,

Zappert calculated that 250 per cubic millimetre was the highest number of eosinophiles which could still be considered normal. As compared with this number, the average of the three cases cited, which works out at 11,000, is nearly fifty times as great. In this way the results of Müller and Rieder's own counts fully confirm Ehrlich's statement.

Since this time a very large number of further observations

have confirmed the correctness of the view that a marked increase of eosinophile cells takes place. In exceptional conditions, however, this increase may be absent, as when the disease is complicated by septic or infective processes, and also in the atypical and acute cases.

At the time when Ehrlich set up the doctrine of the diagnostic importance of eosinophilia in leukæmia, simple eosinophile leucocytosis (see p. 164) had not yet been recognised. This was only found at a later date in connection with asthma, etc. But even this further discovery did not overthrow the correctness of the doctrine. Confusion between those conditions which accompany eosinophilia and leukæmia is quite excluded, since there is not the slightest resemblance between the clinical aspects of these conditions. But apart from this, the appearance of the blood offers plentiful differentiating characteristics. (1) The total increase in the number of white cells rarely reaches a degree which could remind the hæmatologist of leukæmic blood; (2) eosinophile leucocytosis is exclusively polynuclear; (3) mast cells and neutrophile myelocytes are almost completely absent.

A further argument in favour of the diagnostic value of the absolute increase in the number of eosinophile cells is found in those cases which present a blood picture which is extremely like that of leukæmia, but in which the diagnosis of leukæmia can be excluded by the absence of eosinophile cells. An instance of this is found in a case of carcinomatosis of the bone marrow described by Epstein. The blood in this case presented an appearance of anæmia such as is nearly always found in leukæmia, and revealed further an increase of the white cells similar to that seen in leukæmia, with numerous neutrophile myelocytes and nucleated red blood corpuscles. Every one who, like Müller and Rieder, holds that the number of eosinophile cells need not be taken into account in making the diagnosis, would have diagnosed this as a case of myeloid leukæmia. This was, in accordance with Ehrlich's teaching and with the actual state of affairs, excluded by the absence of eosinophile cells.

In view of all these considerations it is advisable, in accordance with Ehrlich's teaching, to regard an absolute increase in the numbers of eosinophile cells as a very important symptom in the diagnosis of leukæmia, which actually belongs to the nature of the disease. Great caution should be exercised in making the diagnosis in the absence of this symptom, and it should always be borne in mind that this indicates a very unusual condition, for which some explanation will have to be found.

4. **The Absolute Increase in the Number of Mast Cells.**—Mast cells are nearly always increased in number in myeloid leukæmia. It is possible to count these elements in leukæmic blood when the films are stained by triacid or by eosin-methylene-blue. When stained by the former they appear like polynuclear non-granulated cells, since their granules do not take on any stain from the triacid mixture, and these cells have therefore been described by Uthemann in his dissertation and classified as non-granulated cells. It was only at a later date that Ehrlich recognised them as mast cells.

The mast cells are more easily recognised after staining with Giemsa, or still better with Jenner's stain, since the granules are not dissolved in the methyl alcohol as they are in watery solutions.

The increase of mast cells is an absolute and very considerable one in nearly every case of myeloid leukæmia. They are usually half as or quite as numerous as the eosinophiles, and at times they may even be present in still greater numbers than the latter. It follows from this that the mast cells increase at a relatively higher rate than do the eosinophiles, as their normal percentage of the total number of the leucocytes is only about 0·28 per cent. The diagnostic value of the increase of these cells in myeloid leukæmia is perhaps even more valuable than that affecting the eosinophile cells, especially because at present no other condition is known in which a marked increase of mast cells is met with.

It must, however, be borne in mind in this connection, that in certain exceptional conditions, such as acute and atypical cases, the increase is usually not present or these cells may be altogether absent from the blood.

5. **Atypical Forms of White Blood Corpuscles.**—These are: (*a*) Dwarf forms of polynuclear neutrophile or eosinophile elements. They were first described in connection with leukæmia by Spilling. As a rule they are merely small specimens of normal polynuclear cells. (*b*) Dwarf forms of mononuclear neutrophile and eosinophile leucocytes. The significance of the dwarf forms of the leucocytes in leukæmia is not yet sufficiently explained, and it is difficult to decide whether they enter the blood as small structures or whether they become smaller in the blood by fission and by constriction. It is, however, more probable that their production was faulty from the beginning, in correspondence with the overproduction of cells. (*c*) Cells showing mitosis. It was formerly believed that the detection of mitosis in leukæmic blood was of considerable importance, since it was held that this phenomenon signified that the increase of the white blood corpuscles took place in the circulating blood as a result of the process of fission. This view was defended more especially by Löwit. A number of authors (H. F. Müller, Wertheim, Rieder) have demonstrated the occurrence of mitosis, more especially of the myelocytes in leukæmia in the circulating blood. The mitosis, however, is not of any diagnostic importance. In the first place, it can only be demonstrated by the application of special methods; and secondly, it is present only in very few cells. Müller stated that he had to examine many thousands of white blood corpuscles to find one single instance. Only in one case did he meet with somewhat more numerous specimens, but even then the proportion was one nucleus undergoing mitosis to several hundred leucocytes.

This find, which must be regarded as practically a negative one, teaches that mitosis only plays a negligible part in the increase of cells in the blood. It is of no value in the diagnosis of leukæmia.

6. **Myeloblasts.**—The blood of every case of myeloid leukæmia contains a certain number of non-granulated cells of the myeloid system,—myeloblasts (Naegeli). These structures were formerly confused with Ehrlich's so-called large mononuclears

(they can be readily distinguished from these by the nucleus) or with the large lymphocytes, or else they were all included in one class. A more exact analysis, however, reveals that they are totally different cells. These cells strike the experienced morphological investigator at once as a special kind of cell, and the marked essential correspondence with the myelocytes is clearly noted. The points which indicate the analogy to the myelocytes are the colour of the staining, the size of the nuclei and its proportion to the protoplasm of the cell. The nucleus usually includes several nucleoli (from two to four), which are well seen when stained by Giemsa. The protoplasm is basophile. At times early granulation of a neutrophilic nature may be seen in these cells, and when stained by Giemsa and triacid stains a large number of every conceivable intermediate form between myeloblasts and myelocytes may be met with.

It is quite clear and obvious for many reasons that these cells are not lymphocytes. In the first place, the development of a neutrophile granulation proves that they cannot be cells of lymphatic tissue, since this tissue is not capable under any circumstances of producing neutrophile granules. In the next place, it would be necessary to ascertain where the lymphocytes could come from, since histological research shows that the lymphatic tissue is eliminated and substituted by myeloid tissue. The final proof against the lymphatic nature of these cells is obtained by staining with Schridde-Altmann's dye mixture. These cells stained in this way do not show any fuchsinophile granulation, which is always present in lymphocytes. There are besides biological reasons for deciding that the non-granulated cells must belong to myeloid and not to lymphatic tissue. These cells increase very extensively immediately before death and in acute exacerbations of the disease, as Ehrlich first noticed and as will be described later. It would be most extraordinary if under such conditions a lymphatic cell production should become prominent. Much more probable would be the production of the least mature and most indifferent form of myeloid cell. This suggestion has actually been made by Türk. However, histolo-

logical tests must decide primarily in such cases, and these tests have decided that lymphatic tissue does not proliferate in myeloid leukæmia, but is crushed out of existence, and that the myeloid character of the proliferation is actually proved by the presence of large numbers of myeloblasts.

A further argument in favour of the view sketched above, and one which in the opinion of the author is very convincing, is that all acute forms of myeloid leukæmia (see p. 191) show high and steadily increasing myeloblast values from the beginning. The sending forth of such an immature medullary cell must therefore be regarded as a sign of exhaustion, and of an absolutely precipitated cell formation of the myeloid tissue.

Ehrlich mentioned these forms of changes in leukæmic blood in the first edition of this work, and pointed out that such occurrences at times might give rise to serious difficulties in the diagnosis. He wrote on page 126 of the first edition of this work :—

Zappert reports the case of a patient who presented the typical appearances of a myeloid leukæmia in February 1892. *Inter alia*, the proportion of the white to the red blood cells was found to be as 1 : 4·92, and 1400 eosinophile cells per c.mm. (3·4 per cent.) were found. The patient was admitted in a very pitiable condition into hospital toward the end of September of the same year and died soon afterwards. During this period of observation the counts showed a ratio of whites to reds of 1 : 1·5 ; a percentage of 0·43 eosinophiles, the majority of the mononuclear cells were free from all traces of neutrophile granulation, and represented about 70 per cent. of the white cells. Zappert emphatically points out that these cells were not in the least like lymphocytes. Zappert found at the post-mortem examination that the bone marrow was infiltrated with a large number of non-granulated mononuclear cells, while the eosinophile cells were considerably less numerous than they usually are in the bone marrow in leukæmia. Dr. Blachstein, under Ehrlich's direction, examined a second case of this kind. The patient had likewise been under careful clinical observation on account of a myeloid

leukæmia for a long time. During his last stay in hospital the examination could only be carried out one day before he died. The death was due to a septic complication. It was found that the blood showed all the marked characteristics of leukæmic blood. There were 62 per cent. polynuclear cells and 17·5 per cent. mononuclear non-granulated myelocytes of about the size of ordinary myelocytes, 0·75 per cent. eosinophile cells, and moderate quantities of nucleated red blood corpuscles. The preponderance of polynuclear and the small number of eosinophile cells was accounted for by the presence of the septic infection; on the other hand, the absence of granules in the mononuclear cells was very curious.

Both these cases can only be adequately explained by presuming that in certain terminal stages the organism loses the power of forming neutrophile substance. Analogous conditions occur in non-leukæmic affections; for example, in a case of post-hæmorrhagic anæmia described by Ehrlich. In such cases it is of great importance to keep in mind these rare cases, which are usually not taken into consideration at all, since this want of knowledge could easily give rise to gross errors with regard to the nature and origin of the mononuclear cells, and might lead to the assumption of a splenic form of leukæmia.

It will be seen how both these investigators adhered to the myeloid character of the blood formation. Since this publication a large number of further observations have been reported in this connection (Naegeli, Hirschfeld, Billings and Capps, Warburg, von Jaksch, Mager and Sternberg), and minute histology, biology, and detailed morphology have proved concurrently that these cells are not lymphocytes, but really myeloblasts.

There still remains one thing to be proved, whether, as Ehrlich and Helly have assumed, the myelocytes have lost their granulations, or whether these myelobasts are to be regarded as a new form of cell, a precursor of the myelocytes. The latter view is the more favoured one at the present date, for these cells cannot be distinguished from the myeloblasts which are normally present in the bone marrow, and the study of the cells themselves

shows that they are young immature cells, because their protoplasm still has a marked basophile reaction, and because granules very frequently appear immature in young forms. This has been observed in cells with commencing neutrophile, and especially well marked in cells with eosinophile, granulations.

7. **Nucleated Red Blood Corpuscles.**—These cells are constantly found in the blood of leukæmia. Their number in the various cases is very variable; at times they are very sparse, and at other times every microscopical field contains numbers of them. The normoblastic type is the most frequent, but this form of cell is not infrequently found in conjunction with megaloblasts and intermediate forms. Mitosis has been described in the nuclei of the red discs by various authors, but this only possesses a small theoretical or clinical significance.

The occurrence of erythroblasts in the blood of leukæmia might be a specific phenomenon of the disease, or only a sign of the anæmia accompanying the leukæmia. The author is inclined to adopt the former view, since such a profuse occurrence of nucleated red cells has never been observed in other forms of anæmia of a similar degree.

These are the individual characters of leukæmic blood, on which the diagnosis of the disease is based. It must, however, still be pointed out that even if each individual factor which has been described may be detected in every case of medullary leukæmia, the manner in which they appear, and their numeric ratio to one another and to the total cells of the blood, vary considerably. Apart from the degree of the increase in number of the leucocytes, one case rarely resembles another as far as the other anomalies are concerned. In one case the blood picture possesses a large mononuclear neutrophilic character; in a second case the preponderance of the eosinophile cells is most striking, and in a third case the nucleated red blood corpuscles predominate. Again, the blood may be overwhelmed by mast cells. This shows that there is such a limitless number of possible combinations that each case must possess its own individual type. It is true that the stages of the disease differ markedly from one

another. For example, the number of myelocytes is smaller at first, and later on increases steadily.

It is of especial importance to study the changes which the blood in medullary leucocythæmia undergoes during the course of an intercurrent disease, and also under the influence of successful treatment by arsenic or Roentgen rays. It has been seen from the exhaustive investigations which have been undertaken by A. Fränkel, Lichtheim, Neutra, Dock, and others on this subject, that the total number of leucocytes undergoes an extraordinary decrease under the influence of febrile conditions. The blood then alters its characters in that the myelæmic type in all its individual details is more and more obscured, and the polynuclear neutrophile elements become markedly predominant. The latter named cells may reach percentages which are usually only seen in ordinary leucocytosis, *e.g.* 90 per cent. or higher.

The organs which form the leucocytes are also changed under these conditions, and their functions become more like their normal functions. But as soon as the action of the foreign stimuli is discontinued the former leukæmic picture rapidly reasserts itself.

In recent years an acute form of myeloid leukæmia has been observed, as well as the ordinary chronic form. A number of undoubted cases of this nature has already been published, *e.g.* by Billings and Capps, Hirschfeld, Naegeli, Benjamin and Sluka, Mager and Sternberg, Ziegler and Jochmann, and others. The clinical appearances have a very close resemblance to that of acute lymphæmia, especially on account of the marked hæmorrhagic diathesis, the great prostration and the not infrequent sudden onset of the disease. The course is often a stormy one. In all undoubted cases of this kind the blood shows characters which differ considerably from that which is seen in the ordinary form. It can be recognised at once that the condition is abnormal and unusual. Corresponding to the rapid proliferation of the cells, numerous myeloblasts appear in the blood from the beginning, as the most indifferent, lowest type of cellular

elements, and their number generally increases relatively and absolutely with surprising rapidity. Nucleated red blood corpuscles are very often present in large and even in enormous numbers.

Certain atypical conditions are frequently noted, in the blood in connection with this rapid proliferation. The most common variation is the sparse presence or even absence of the eosinophiles and of the mast cells. This is, however, not regular. A few observations, including one of the author's, revealed very high values for the acidophile cells.

Histologically, this form of myeloid leukæmia does not show any special characteristics, as would have been expected, save that the organs contained very large numbers of myeloblasts.

It is possible to form quite clear conceptions with regard to the nature of the leukæmic conditions. It is true, the cause of the affection is still unknown; but the affection itself is characterised by an enormous proliferation of myeloid tissue in all situations in which such tissue is formed and increased. The whole bone marrow is filled with functionating medullary cells in the first place. In the next place, as in anæmias and infectious diseases, foci are formed in the splenic pulp, in the centres of the lymphatic glands, in the liver, in the omentum, and in short everywhere, but always in intimate association with blood vessels. According to Marchand and Naegeli, this would indicate a differentiation of the adventitial cells into myelocytes, and a development of cells which had hitherto remained undifferentiated; while, according to Schridde, the cells of the vascular wall, *i.e.* the endothelial cells, which had retained their embryonal type, would develop, just as during the embryonal period, to erythroblasts, myeloblasts, and myelocytes. In any case, the formation only takes place locally, and in close association with the blood vessels. Cells of the bone marrow type can never be formed from other kinds of cells, such as lymphocytes. This metaplasia, or, in Schridde's language, heteroplasia, cannot be regarded as a tumour-like process, or

like a sarcomatosis; for the same formations are found in many forms of anæmia, infectious diseases, and to a vast extent, for example, in the curable pseudo-pernicious anæmia of infants. This disease is therefore a pathological but not a tumour-like proliferation of the myeloid tissue. This proliferation is met with in very varied affections, but in its highest development in leukæmia.

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CHAPTER IV

THE BLOOD PLATELETS: HÆMOCONIA

A THIRD formed element of normal blood, the blood platelets, was first described by Hayem and later by Bizzozero. These cells are round or oval discs without any hæmoglobin. The form is extremely susceptible to mechanical, thermic, and chemical influences. These cells measure about 3 μ in diameter. A marked characteristic of these cells is the tendency to form largish clump "bunches of grapes," which is due to their extraordinary stickiness. This peculiarity renders it particularly easy to distinguish them from other formed elements of the blood, but at the same time it renders all exact study and counting extremely difficult. When the apparatus which is usually employed for counting blood cells is used to count these cells the results are quite unreliable, since the blood platelets rapidly adhere to the walls of the vessels. All the earlier authors attempted to get over this difficulty by employing a special diluting fluid (14 per cent. magnesium sulphate solution—Bizzozero), which prevents the clumping of the platelets, but a few cells adhered even to the glass walls of the capillary tube in which the dilutions are made.

Brodie and Russell recommended a mixture in which the blood platelets remained permanently isolated and in which they could be stained. They allow a drop of blood to fall directly into a drop of the solution and then determine the relative proportions of red cells to blood platelets. Their solution was made up as follows:—

> Dahlia glycerine, and
> 2 per cent. sodium chloride solution, in equal parts.

Sahli added a sufficient quantity of methyl-violet to Bizzozero's magnesium-sulphate solution to make a perfectly clear fluid when

placed in a 10 c.c. measure cylinder, and allowed a drop of this fluid to fall on the skin. He pricked the skin through the drop, so that the blood on issuing from the prick mixes at once with the solution. He then determined the relative proportions of the platelets to the blood corpuscles. Helber used a freshly prepared 10 per cent. solution of sodium meta-phosphate to dilute the blood.

Another method is the relative counting of the blood platelets in stained dry films. This method has been employed by the majority of observers in recent times. Ehrlich found that in specimens treated by the iodine eosin method (see p. 53) the blood platelets are conspicuous by their intense red colour, which corresponds to the high alkali content of these elements, and in this way they can be easily counted.

Bürker recommended a sort of accumulation method for the purpose of obtaining these cells for examination. He allowed a drop of blood to fall on a smooth surface of paraffin, and then placed the paraffin in the moist chamber. The heavier erythrocytes and leucocytes soon sedimented, while nearly all the blood platelets were found on the surface of the drop after about twenty to thirty minutes. They were easily picked up on to a cover glass, and then examined directly.

Levaditi, Rosin and Bibergeil, Puchberger, and later on a large number of other observers employed another method. A drop of a weak alcoholic solution of a dye (*e.g.* brilliant cresyl-blue) was allowed to dry on a cover-slip, and the blood was received directly on this cover-slip. More recently Romanowsky-Giemsa's staining has been employed with good results for the study of blood platelets.

No reliable results have as yet been obtained in the counting of these elements, either with regard to their relative or their absolute numbers. The normal values have been fixed at 200,000 to 300,000 by Ebner, 635,000 by Brodie and Russell, 730,000 to 962,000 by Kemp. From this it will be seen how little value can be attached to counts in cases of disease at present, and consequently how misleading any deduction must be when based on such counts.

Deetjen has described a special method of studying the platelets. "5 grms. of agar-agar are dissolved by boiling for a half-hour in 500 grms. of distilled water, and the solution while still hot is filtered through a folded filter paper, through which it passes readily without using a hot filter funnel. 0·6 grm. of NaCl, 6 to 8 c.c. of a 10 per cent. solution of $NaPO_3$, and 5 c.c. of a 10 per cent. solution of K_2HPO_4 are added to each 100 c.c. of the filtrate. For the examination of the blood a little of the agar solution is poured on to a slide and allowed to set. After the agar is quite cold a strip about 2 mm. broad is cut out of it, and the drop of blood gained from the finger is applied to this strip. This is then covered with a cover-slip."

The amœboid movements of the platelets may be seen by means of this method, and they can thus be more closely studied than in any other way.

With regard to the origin and significance of the blood platelets, the majority of authors (of whom Hayem, Bizzozero, Laker, and Arnold should be especially mentioned) have come to the conclusion that they are preformed in living blood. The author believes that this view is correct. The opposite view, which is held especially by Löwit, that these structures are formed in the blood after it has left the blood vessels, is denied by the author, on the ground of his own extensive observations.

Some authors, including Deetjen, Argutinsky, and others, have, within recent times, suggested that the blood platelets should be regarded as independent complete cells. This opinion is based on the generally accepted structure of the majority of the platelets, which at times even in unstained specimens show a strongly refractile internal substance and a less strongly refractile external substance; this division is confirmed by the tinctorial behaviour of the elements. The internal substance stains with strong basic dyes and when nuclear dyes are applied in strong solution (Pappenheim).

Morawitz deduced from the part which the platelets play in the coagulation of blood (see below), and from their thrombogen

content which places them in a different category to all other elements of the blood, that they must be independent cells.

These facts, however, are insufficient to show that the platelets should be regarded as real cells. The chief objection to this view, which has been held by other and especially the older authors, is an observation of Schwalbe's. He found these platelets especially numerous in tied vessels between the two ligatures.

Whether they are intravital fragments of plasmatic substances or whether they are cast off from the cells cannot at present be decided with certainty, even if there is reason to believe that the latter view is probably correct. The glycogen content of the platelets (see p. 52) certainly suggests that they are derivatives of the blood cells.

Some authors believe that the platelets are derived from the leucocytes (F. Müller, Arnold, Grawitz). The most striking argument against this supposition is the fact, which was demonstrated by Helber, that in the blood of mammalian embryos, platelets can be found before the leucocytes have been formed. If the platelets were derived from leucocytes it would be necessary to assume that they are formed in a different manner during embryonal life than during post-embryonal life, or that a second method of production comes into force after birth.

The suggestion that the blood platelets are derived from the red blood corpuscles receives more support at present than any other suggestion. There are, however, three different conceptions with regard to this mode of origin.

According to Arnold, Schwalbe, and their pupils, both pigmented and pigment-free plates originate by means of erythrorrhexis and erythroschisis. Great caution is necessary with regard to the acceptation of this supposition, since it is scarcely possible to differentiate between schistocytes and platelets, and since the complicated structure of the platelets would be difficult to account for if this were true.

The last-mentioned objection would also apply to the suggestion made by Weidenreich, that the blood platelets are detached particles from the surface of the erythrocytes.

The so-called nucleoid theory appears to be well founded, and is supported by the majority of hæmatologists. According to this theory, the blood platelets owe their origin to the remains of nuclear substance, which result from the endo-corpuscular karyolysis of the erythrocytes. This theory has recently received considerable support from the observations made by its founder, Pappenheim, with the assistance of the dark field microscope. Blood platelets are, according to this theory, nothing else than discharged nucleoids. One or two very striking facts speak greatly in favour of it. These include the staining of the chromatin and also direct morphological analysis. Not infrequently the specimens give the appearance as if the blood platelets completely formed were issuing from the bodies of the red blood corpuscles (Köppe, Engel, Maximow, Hirschfeld). The pictures seen in these specimens are often so suggestive that Naegeli's objection can scarcely lessen the likelihood of the correctness of the doctrine. Naegeli was of opinion that the specimens seen by him were merely instances of blood platelets superimposed on the middle or edges of erythrocytes.

A view which is held by a number of observers (Schwalbe, Grawitz), that the platelets may be derived from various sources, is the least likely of all. If it were accepted it would be necessary to give up regarding the platelets as uniform elements of the blood.

J. H. Wright has quite recently observed the production of the blood platelets in bone marrow and in the spleen; he has seen them being formed by detachment of the plasma of megakaryocytes.

The knowledge possessed at present with regard to the physiological function of the blood platelets is also extremely defective. The original view which was put forward by Hayem, that the platelets are the precursors of the red blood discs and should therefore be called hæmatoblasts, is regarded by the majority of hæmatologists as untenable. On the other hand, the intimate connection between the blood platelets and coagulation which was first noticed by Bizzozero has been recognised in

nearly all the recent works on the subject (cf. Löwit and Schwalbe's reviews). It is still uncertain whether the substance of the platelets yields the material for the formation of fibrin as Bizzozero suggests, or whether they only play the part of intermediators, in accordance with the observations of Eberth and Schimmelbusch on the formation of thrombi. It would occupy too much space to enter into a discussion of the chemical aspect of this question in this place, and for this reason only a few clinical observations will be mentioned, which point to the relations between the coagulability of the blood and the platelet content.

A considerable increase of blood platelets is found especially in chlorosis (Muir), and also in post-hæmorrhagic anæmia (Hayem). In both these conditions there is a marked raising of the coagulability of the blood. An important observation of Denys should, however, be mentioned, who found that in two cases of purpura the only morphological change of the blood was a very considerable decrease of blood platelets. It is well known that the coagulability of the blood is markedly diminished in purpura, or may be abolished altogether. Ehrlich also was able to examine a similar case, in which the blood platelets were entirely absent.

A number of authors (Cesaris-Demel, Hayem, Levaditi, Rowley) have described an increase in volume of the platelets in various anæmias, while Pappenheim described the same in polyglobulæmia. The size may be as large as that of a normal erythrocyte.

Le Sourd and Pagnier have suggested another method of gaining information with regard to blood platelets. By injecting the blood platelets of rabbits into guinea-pigs they obtained a serum from the guinea-pig which was capable of exercising a specific action on the rabbit's platelets. This serum destroyed the platelets *in vitro*, and in the body of the living rabbit it caused them to disappear altogether without in any way damaging the erythrocytes or leucocytes. The deduction which these authors drew from these experiments, that the platelets could not

be derivatives of either red or white blood corpuscles, does not appear to be justified; it could be assumed that the platelets, being the products of disintegration, would possess a smaller or different kind of resistance to that of the mother cells.

Gruber and Futaki extracted a substance from blood platelets which acted bactericidally against tetanus. Tschistowitsch is inclined on the strength of his blood platelet counts to apply this observation generally, and to regard these elements as being possessed of the function of carrying the protective substances of the blood.

Ottolenghi regards the blood platelets as the originators of alexin, because he ascribes to them the capability of reactivating donkey's or rabbit's serum, which had been robbed of a bactericidal action towards tetanus bacilli by heat.

H. F. Müller has described a fourth element of blood, and has given it the name of hæmoconia. These are found in blood plasma in the form of very minute, colourless, highly refractile corpuscles, like granules or cocci. They are possessed of active molecular movement, which can be watched for a very long time without any special precautionary measures. They do not turn black with osmic acid (Müller), and therefore do not contain fat. They do not appear to have any connection with the formation of fibrin, since they are always found outside the fibrin network. Müller found them in every specimen of normal blood, but noted that their number varied very considerably. They were very markedly increased in number in one case of Addison's disease, and diminished in starvation and in cachexia.

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DESCRIPTION OF PLATES

DESCRIPTION OF PLATE I

(Magnification, 700 Diameters)

1–6.—**Myeloblasts** (Giemsa staining).

Cells 1–3 are from a case of chronic myeloid leukæmia.

Cells 4–6 from a case of acute myeloid leukæmia.

The nucleus corresponds to the nucleus of a myelocyte, showing a delicate chromatin structure and three or four distinct blue nucleoli. The protoplasm possesses a basophile reticulum, reaching right up to the nucleus. There are no granules.

7–9.—**Development of Myeloblasts to Neutrophile Myelocytes** (Giemsa).

Cell 7 (chronic myeloid leukæmia). The reticulum is still markedly basophilic; there are but few granules, and the nucleoli are still visible.

Cell 8 (chronic myeloid leukæmia). Similar to No. 7. The nucleoli are still distinct, but the granulations are more plentiful.

Cell 9 (another case of chronic myeloid leukæmia). No nucleoli are visible; the granulation is very sparse.

10–17.—**Neutrophile Myelocytes** (Giemsa).

Cells 10–14 and 17 are from cases of myeloid leukæmia.

Cells 15–16 are from croupous pneumonia.

The granules are very numerous. The basophilic character of the protoplasm has diminished.

Cell 17, slightly crushed (demonstration of isolated granules).

18.—**Metamyelocytes (Leukæmia)** (Giemsa).

Transition of myelocyte nucleus to polymorphous form.

19–25.—**Polymorpho-nuclear Neutrophile Leucocytes** (Giemsa).

Normal blood and leucocytosis.

Cell 25 crushed (isolated granules).

26–30.—**Eosinophile Myelocytes** (Giemsa). From two cases of chronic myeloid leukæmia.

Cell 26.—Nearly all the granules are blue (basophile preliminary stage). At the edge some red granules are seen.

Cells 27–28 and 30 (crushed). The red granules are predominating; only a few blue granules are present.

Cell 29.—All the granules are red (oxyphile, ripe), but the protoplasm is distinctly reticulated blue.

31–35.—**Eosinophile Leucocytes** (Giemsa).

Cell 31.—Metamyelocyte from a case of myeloid leukæmia. The nucleus is just taking on the polymorphous structure.

Cell 25 (crushed). Showing isolated granules.

DESCRIPTION OF PLATE II

(Magnification, 700 Diameters)

36–43.—**Mast Myelocytes** (Giemsa). From a case of chronic myeloid leukæmia with very numerous mast cells.

Cell 36.—The blue precursors of the mature granules are differentiated in the protoplasm.

Cell 37.—Numerous blue granules.

Cells 38–40.—A mixture of immature blue and mature mauve-coloured granules.

Cells 41–42.—Mauve-coloured granules,—markedly resistant to water.

Cell 43.—Mature mauve-coloured granulation; no longer resistant to water.

44.—**Mast Cell Myelocyte** (Giemsa). From case of myeloid leukæmia.

45–48.—**Mast Leucocytes** (Giemsa).

The granules are readily soluble in water.

Cells 45–46.—Normal blood.

Cells 47–48.—Chronic myeloid leukæmia.

49–51.—**Mast Leucocytes** (May Grünwald staining). From a case of myeloid leukæmia.

52–57.—**Large Mononuclear Leucocytes and Transition Forms** (Giemsa).

Normal blood and leucocytosis. The granulation is very fine and plentiful, and the protoplasm slate coloured. The series shows a gradually increasing transformation of the nucleus. The much more intense nuclear staining and the much finer granulation than in the myelocytes should be noted.

58–62.—**Stimulation Forms = Pathological Myeloblasts** (Giemsa).

Vacuoles in the deep blue protoplasm.

Cells 58–59.—From a case of pernicious anæmia.

Cells 60–62.—From a case of encephalitis (child aged six years).

63–87.—**Lymphocytes** (Giemsa).

Cells 63–69.—Normal blood, 63–65 without azure granules. 66–69 with azure granules.

Cell 69 somewhat crushed.

Cells 70–72.—Somewhat larger lymphocytes (four years' old child).

Cells 73–87.—Lymphocytes from a case of lymphatic leukæmia.

Cells 73–75.—Nucleus almost free.

Cells 76–81.—Large forms. 78 crushed. 79–81 with azure granules.

Cells 82–87.—Transformation of nucleus into Rieder's form.

DESCRIPTION OF PLATE III

(Magnification, 700 Diameters)

88–97.—**Erythroblasts** (Giemsa). From a case of carcinoma of the bone marrow.

Cells 88–90.—Megaloblasts, showing polychromatic protoplasm.

Cells 91–93.—Intermediate forms between megaloblasts and normoblasts; two are almost orthochromatic.

Cells 94–95.—Polychromatic and orthochromatic normoblasts.

Cells 96–97.—Dissociation of nucleus in polychromatic and basophilic granulated cells.

98–105.—**Erythrocytes** (Giemsa).

Megalocytes and normocytes in all stages from marked polychromasia up to orthochromasia. Case of carcinoma of the bone marrow.

106–118.—**Mitosis of Erythroblasts** (Giemsa).

All the cells possess characteristic basophile granulation of the protoplasm.

Cells 106–115.—All stages of mitosis, from a case of infantile pseudo-leukæmic anæmia.

Cells 116–118.—A typical pathological mitosis, from a case of acute myeloid leukæmia.

Cell 118.—Triple mitosis and triple division of the nucleus.

119–122.—**Remains of Nuclei and Nuclear Debris** (Giemsa). From a case of infantile pseudo-leukæmic anæmia.

Cells 119–121.—Solution of the nuclear remains.

123–142.—**Ring Bodies** (Giemsa). At times with red or blue, or red and blue granulations. The rings are in part free in the plasma.

Cells 123–134.—From a case of pernicious anæmia.

Cells 135–142.—From a case of acute myeloid leukæmia.

By an unfortunate mistake, which could not be corrected after it was discovered, the cells of the series 88–93, as well as cells 102 and 103, were reproduced on a smaller scale than in the drawings. This reduction is considerable and likely to mislead. The diameter of the cells should be about one-third larger than they appear.

DESCRIPTION OF PLATE IV

(Magnification, 700 Diameters)

143–156.—**Red and Blue Punctation in Erythrocytes** (Giemsa).

Cells 143–153.—From cases of pernicious anæmia.

Cells 154–156.—From cases of acute myeloid leukæmia.

A mixture of the blue and red punctation is at times seen, while at other times only the red appears. Two cells show a diffuse red coloration of the protoplasm. The size of the red granules varies at times.

157.—**Red Punctation in Erythrocytes** (Giemsa). From a case of pernicious anæmia.

The cells show very well-marked red granules, singly or in numbers. In the middle there is a polychromatic megalocyte with numerous red granules.

158–169.—**Blue Basophile Punctation** (Giemsa).

Lead poisoning. All the cells depicted are orthochromatic.

Cells 163–169.—Severe chlorosis in the stage of recovery.

There are numerous polychromatic cells with blue basophile punctation. One red grain is also seen.

DESCRIPTION OF PLATE V

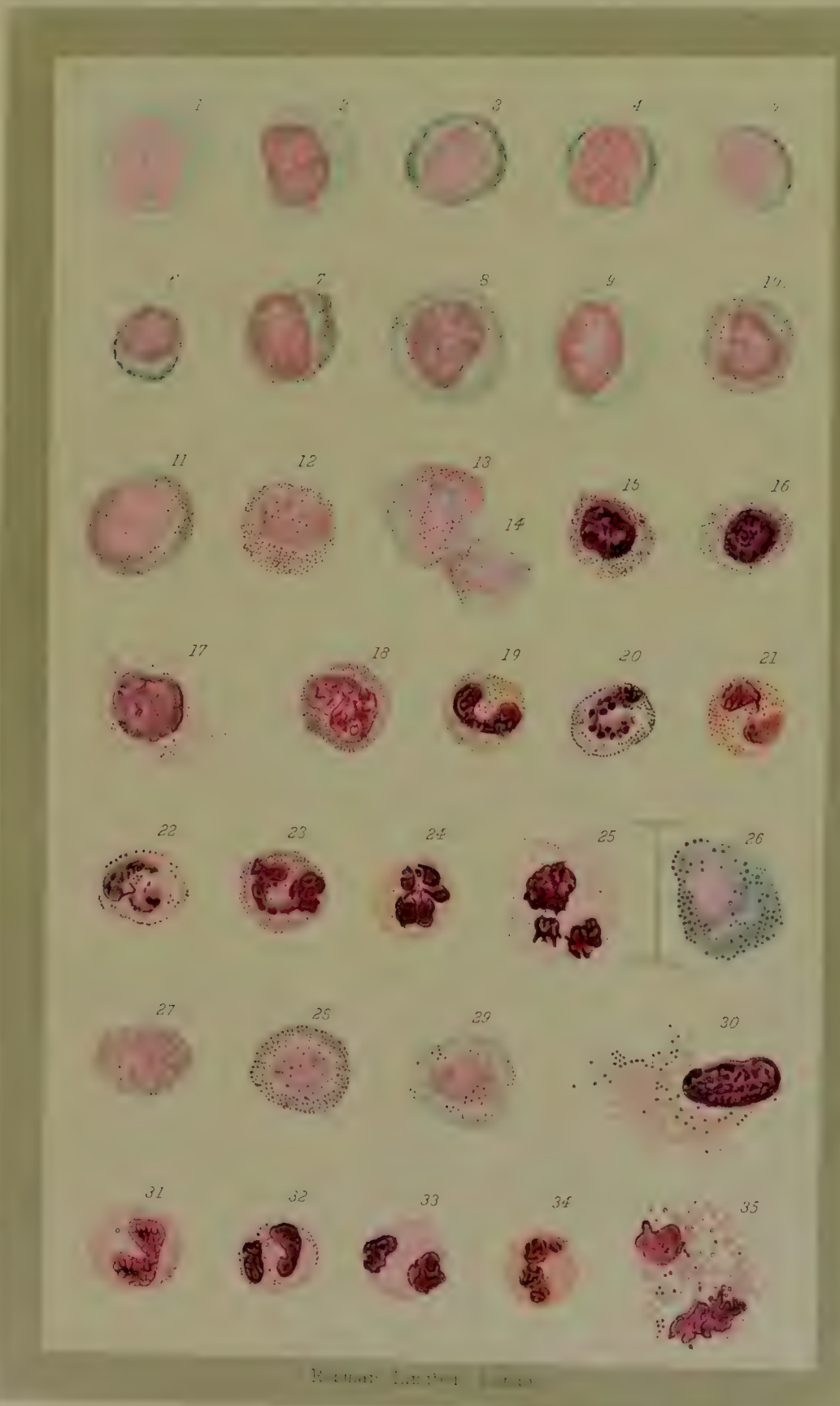
(Magnification, 700 Diameters)

170.—**Blood Platelets** (Giemsa). From the blood in chlorosis.

a–g. **Cells stained by Triacid Solution.**

(*a*) Neutrophile myelocytes.
(*b*) Polynuclear neutrophile leucocytes.
(*c*) Eosinophile cells.
(*d*) Mast cells.
(*e*) Normoblasts.
(*f*) Megaloblasts.
(*g*) Erythrocytes.

The illustrations 1–170 have been drawn in colours by the academic painter, Mr. L. Schrötter (Zurich-Heidelberg), from preparations supplied by Dr. Naegeli, and under his supervision.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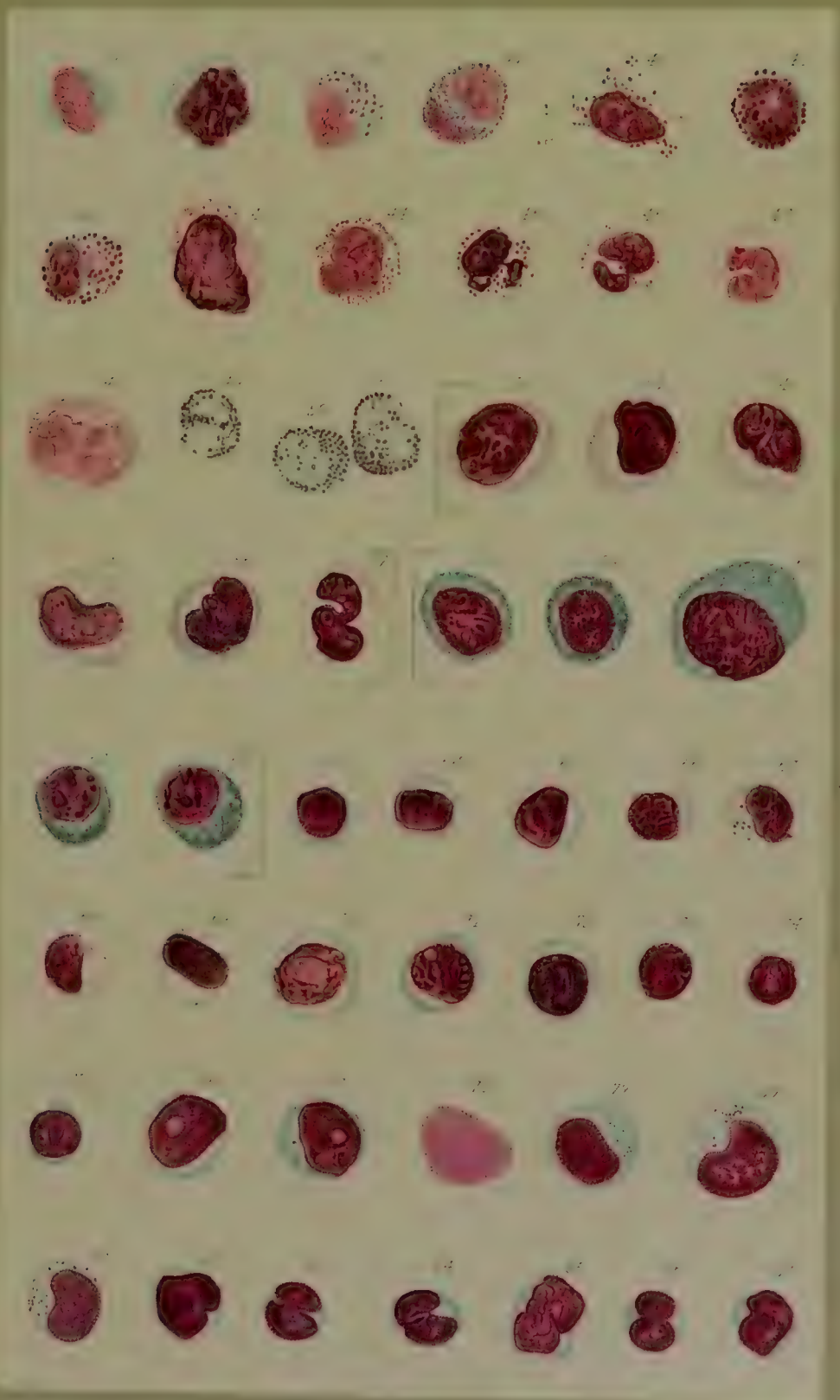

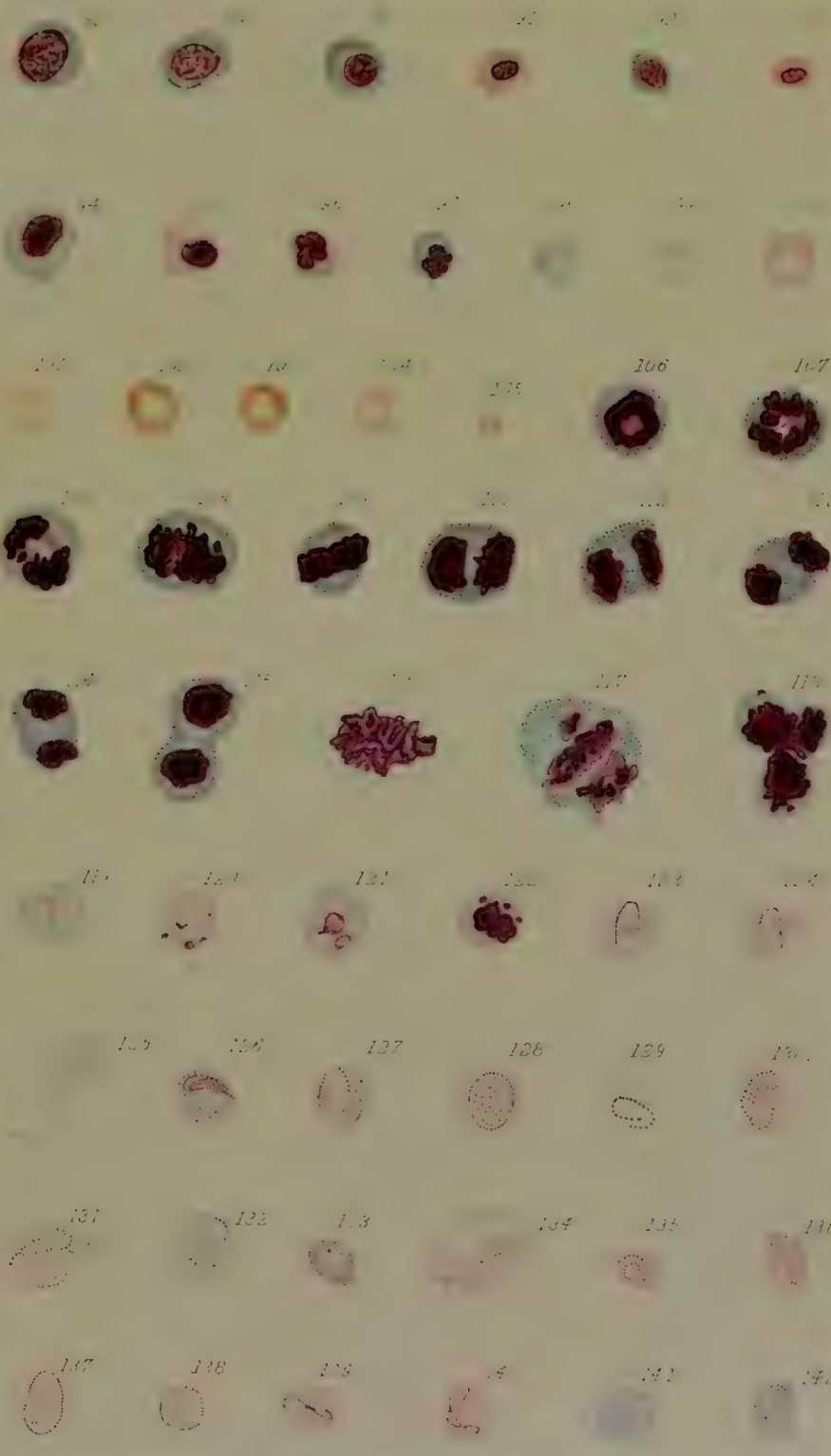

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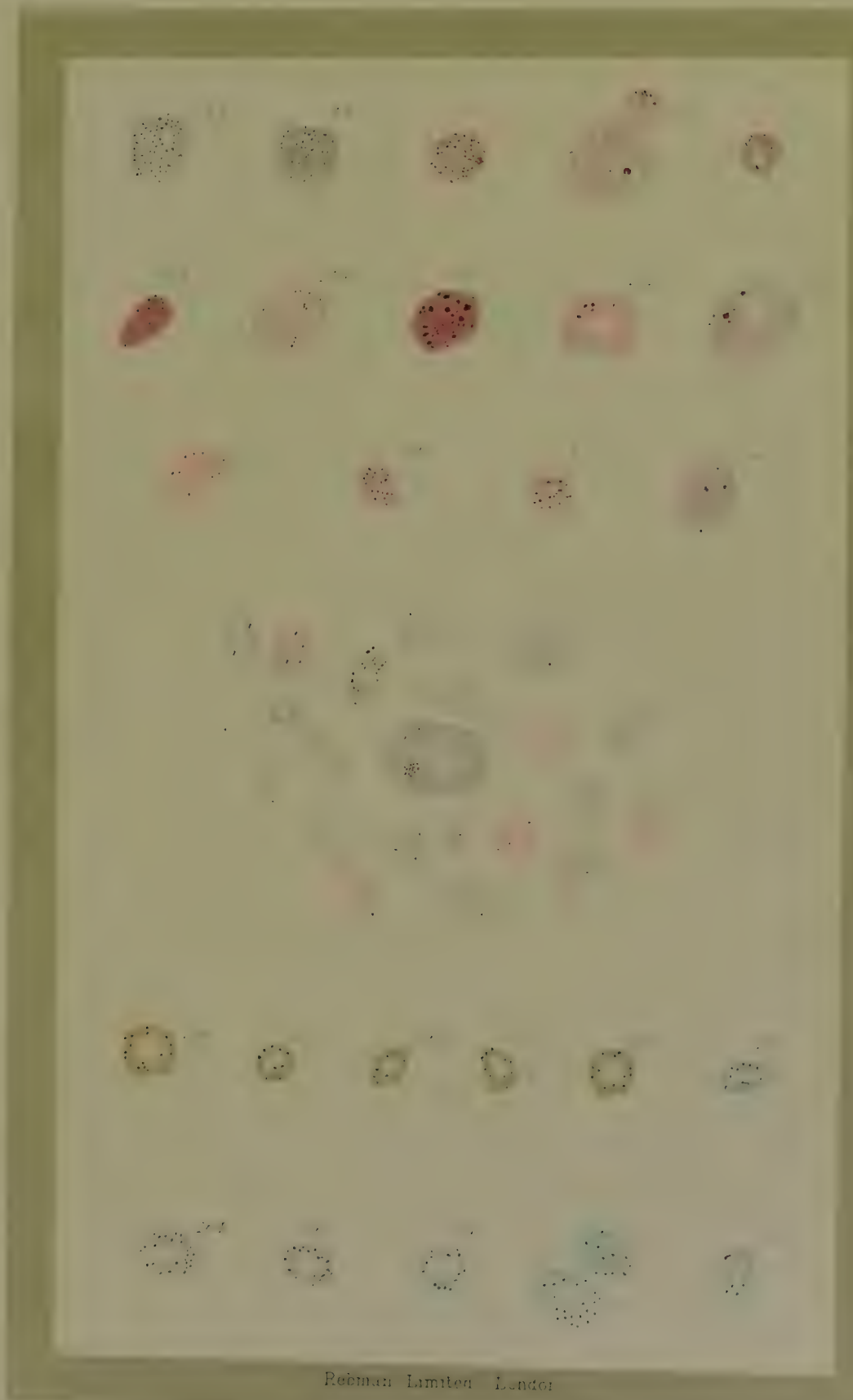

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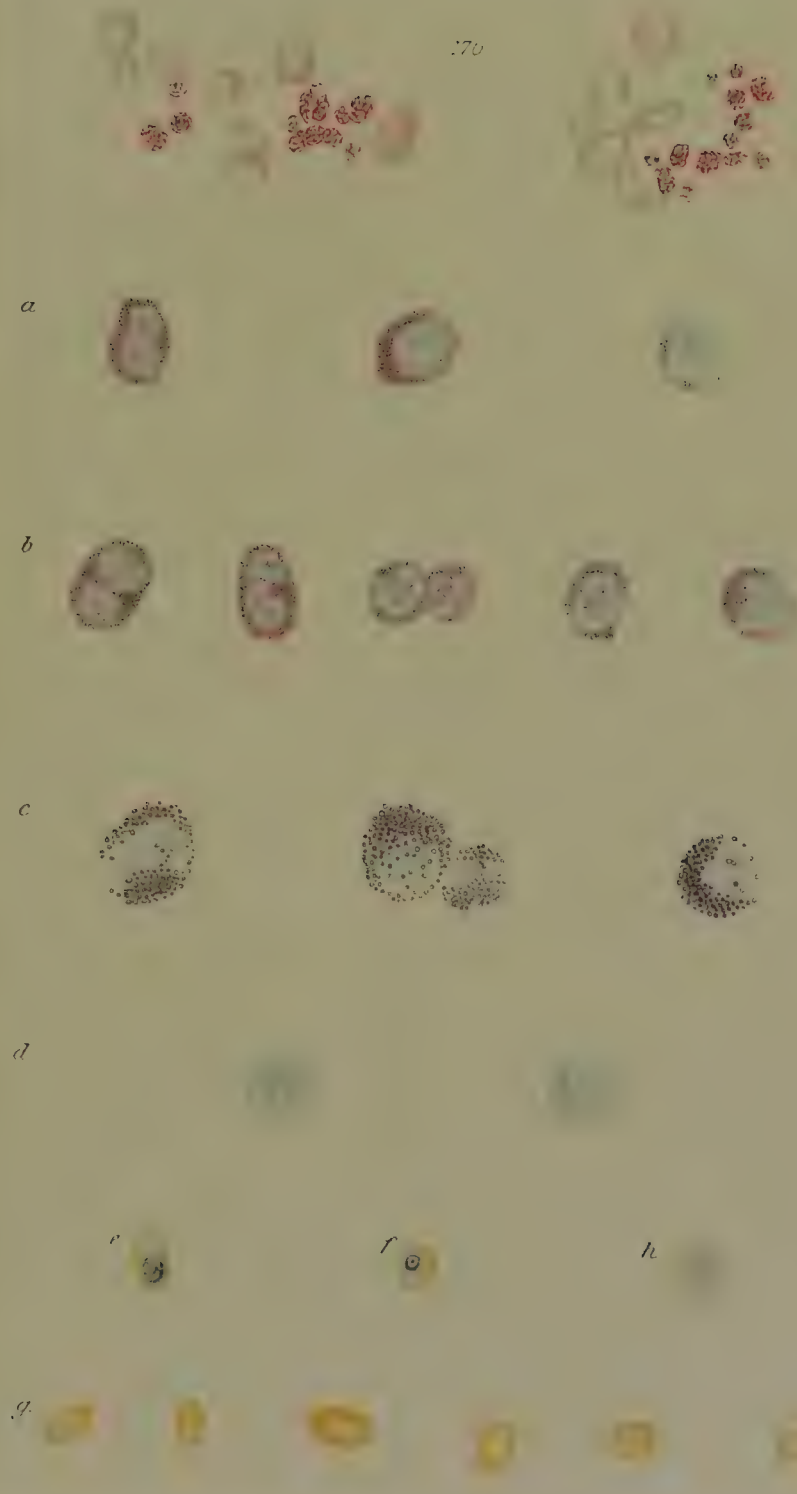

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